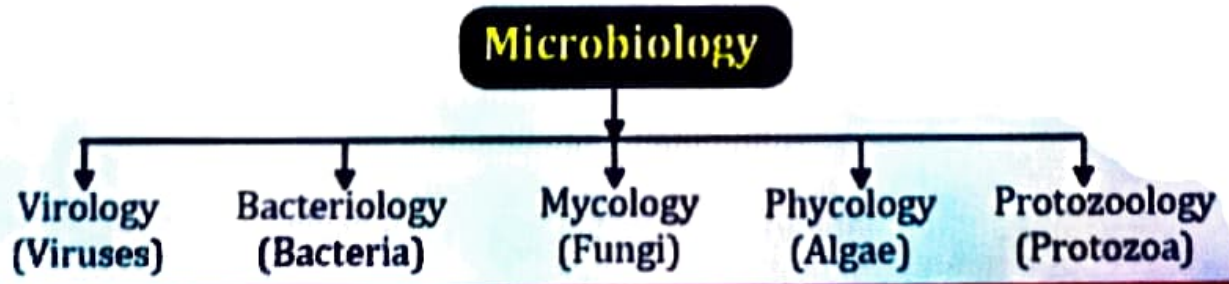
UNIT- I
(CHAPTER-1)
INTRODUCTION OF MICROBIOLOGY

Points to be covered in this topic

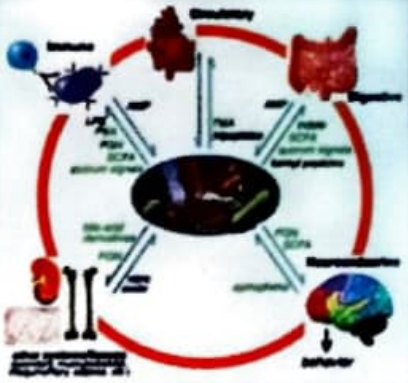
1. INTRODUCTION
2. HISTORY OF MICROBIOLOGY
3. BRANCHES OF MICROBIOLOGY
4. SCOPES OF MICROBIOLOGY
5. IMPORTANCE & APPLICATION OF MICRO.

❑ INTRODUCTION

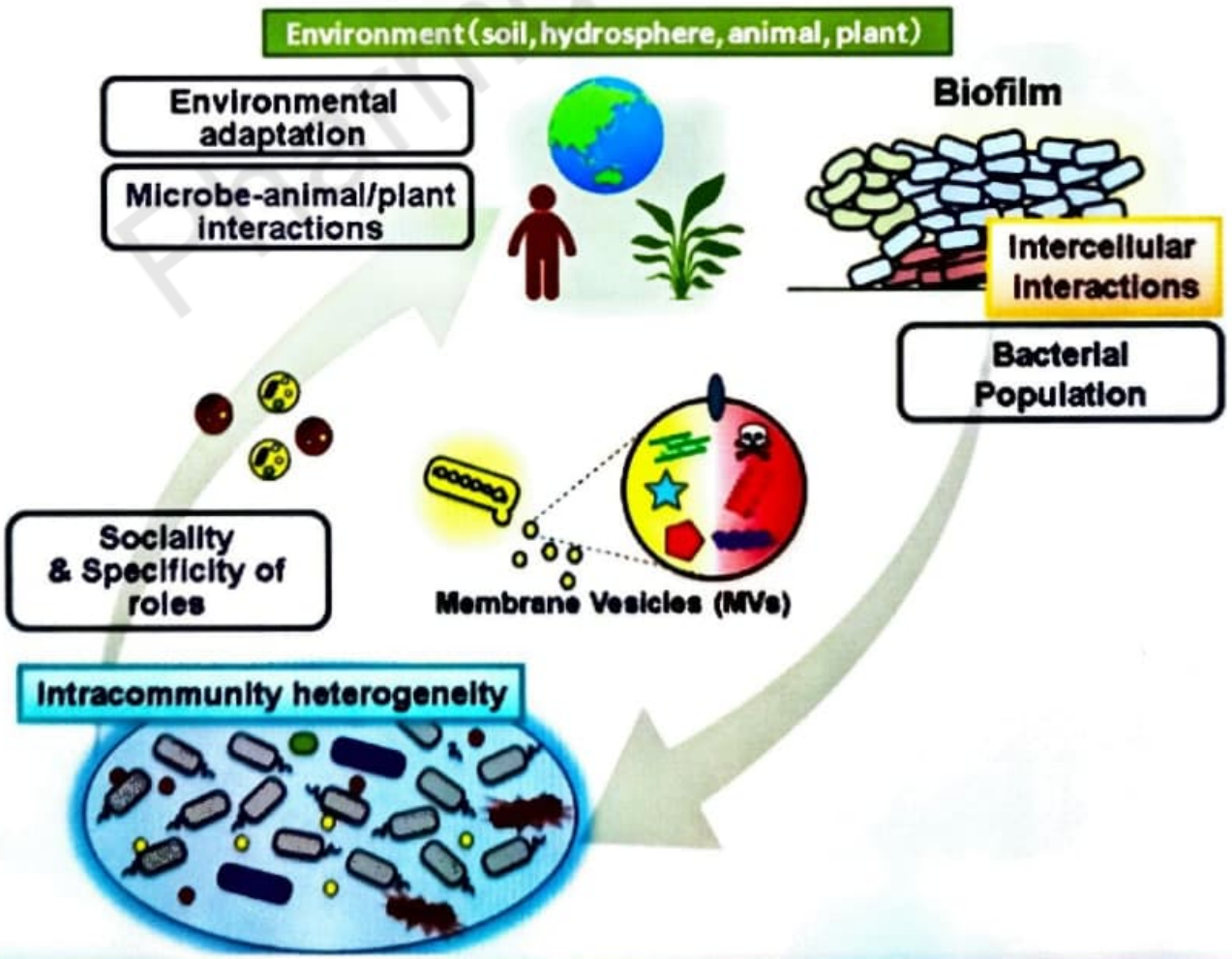
- Microbiology is the **study of microorganisms** that is the organism which are of **microscopic dimensions.** These organisms are too small to be clearly perceived by the **unaided human eye.**
- Microorganisms are living organisms that are **usually too small to be seen** clearly with the naked eye. An organism with a diameter of **1 mm or less are microorganisms** and fall into the broad domain of microbiology.
- At present there is general agreement to include **five major groups** as microorganisms. **The subdivisions are :**



- Microorganisms are relevant to all of us in a **multitude of ways.**
- The influence of microorganism in human life is both **beneficial** as well as **detrimental** also. For example microorganisms are required for the **production of bread, cheese, yogurt, alcohol, wine, beer, antibiotics.**



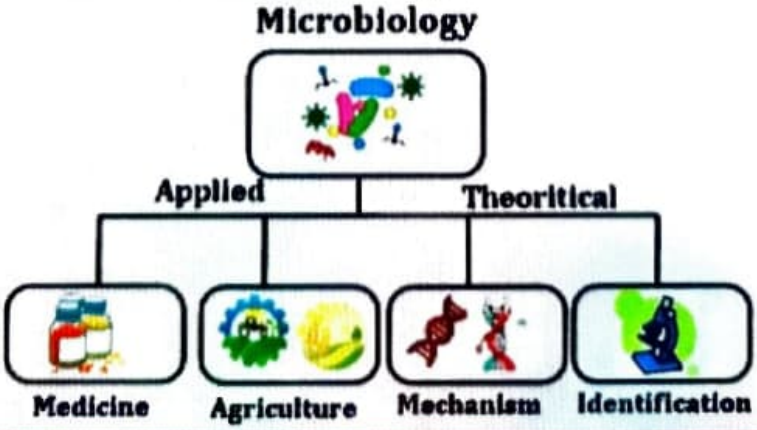
- (e.g. Penicillin, Streptomycin, Chloromycetin), vaccines, vitamins, enzymes and many more important products.
- Microorganisms are indispensable components of our **ecosystem.**
- Microorganism play an important role in the **recycling of organic and inorganic material** through their roles in the C, N and S cycles, thus playing an important part in the **maintenance of the stability of the biosphere.**
- They are also the source of nutrients at the base of all **ectotropical food chains and webs.** In many ways all other forms of **life depend on the microorganisms.**



❖ MAJOR FIELDS OF MICROBIOLOGY

FIELD	DESCRIPTION
Algology, Phycology	Study of algae
Bacteriology	Study of bacteria
Virology	Study of viruses and viral diseases.
Protozoology	Study of protozoans (animal like single celled eukaryotic organisms).
Mycology	Study of fungi (achlorophyllous, heterotrophic, eukaryotic with a rigid cell wall containing chitin/cellulose)
Parasitology	Study of parasitism and parasites (include pathogenic protozoa, helminthes worms and certain insects).
Nematology	The study of nematodes.
Microbial ecology	Study of interrelationships between microbes and environment.
Microbial morphology	Study of detailed structure of microorganism.
Microbial taxonomy	Concerned with classification, naming and identification of microorganism.
Microbial Physiology	Study of metabolism of microbes at cellular and molecular levels.
Microbial genetics and Molecular Biology	Study of genetic material, structure and function and biochemical reactions of microbial cells involved in metabolism and growth.

- The branch microbiology has two major aspects: the **theoretical** and the **applied. Doctors and farmers are applied microbiologist.** For example the doctors has the primary interest to keep people healthy through the use of scientific knowledge while the **scientist (theoretical) work** is to obtain new information in his related field and guide the farmers to **increase crop yield.**



❖ FIELDS OF APPLIED MICROBIOLOGY

FIELD	APPLIED AREAS
Air Microbiology	Deals with the role of aerospora in contamination and spoilage of food and dissemination of plant and animal diseases through air.
Agricultural Microbiology	Study of relationships of microbes and crops and on control of plant diseases and improvement of yields.
Aquatic Microbiology	Study of microorganisms found in fresh estuarine and marine waters.
Biotechnology	Scientific manipulation of living organisms especially at molecular and genetic level to produce useful products.
Dairy Microbiology	Deals with production and maintenance in quality control of dairy products.
Exomicrobiology Medical Microbiology	Deals with exploration for microbial life in outer space. Causative agents of disease, diagnostic procedure for identification of causative agents, preventive measures.
Food Microbiology	Deals with interaction of microorganisms and food in relation to food' processing, food spoilage, food borne disease and their prevention
Industrial Microbiology	Concerned with industrial uses of microbes in production of alcoholic beverages, vitamins, NH_2-acids, enzymes, antibiotics and other drugs.
Immunology	Deals with the immune system that protects against infection and to study serology reactions.
Public Health Microbiology	Concerns with monitoring, control and spread of diseases in communities.

➢ Harmful Microorganisms

- Disease and decay are neither inherent properties of organic objects, nor are caused by physical damage, it is microorganisms that bring about these changes.

- We are surrounded by **bacteria, virus,** and **fungi.** Many microorganisms cause diseases in cattle, crops and others are known for entering human bodies and causing various diseases.

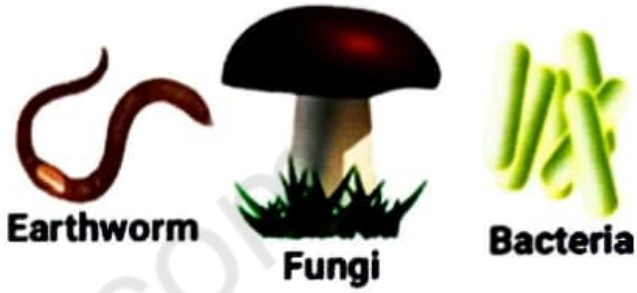
Examples of familiar human diseases are:

Bacteria: pneumonia, bacterial dysentery, diphtheria, bubonic plague, meningitis, typhoid, cholera, salmonella, meningococcal.

Virus: Chickenpox, measles, mumps, German measles, colds, warts, cold sores, influenza.

Protozoa: amoebic dysentery, malaria,

Fungi: Ringworm, Athlete's foot.



➢ Useful-Microorganisms

- As **decomposers**, bacteria and fungi play an important role in an ecosystem. They **break down dead or waste organic matter** and release inorganic molecules.
- **Green plants** take these nutrients which are in turn consumed by animals, and the products of these plants and animals are **again broken down by decomposers**.
- **Yeast is a single-celled fungus** that lives naturally on the **surface of the fruit.** It is economically important in bread-making and brewing beer and also in the making of **yoghurt.**
- Most microorganisms are **unicellular**; if they are multicellular, they lack highly **differentiated tissues.**
- There fundamentally two different types of cells, One being **Prokaryotic** and the other **Eukaryotic**.
- Microbes especially **prokaryotes** are numerous in number in comparison to eukaryotes.
- The lineage of life on Earth originated from these microbes :

1. Bacteria **2. Archaea** **3. Eucarya**

❑ HISTORY OF MICROBIOLOGY

- **Physics began in ancient times**, mathematics even earlier, but the knowledge of tiny living things, their biology, and their impact on human lives have only been around since the late **19th century.**
- Until about the **1880s**, people still believed that life could form out of **thin air** and that **sickness was caused by sins or bad odors.**
- Opinions about why diseases afflicted people differed between cultures and parts of society and the treatments differed as well. **Diseases were thought to be caused by**
  - ✓ **Bad smells**, treated by removing or masking the offending odor
  - ✓ An **imbalance in the humor** of the body, treated with **bleeding, sweating,** and **vomiting**
  - ✓ **Sins of the soul**, treated with **prayer and rituals**
- Although the concept of contagion was known, it wasn't attributed to tiny living creatures but to **bad odors or spirits, such as the devil.**
- **Varo and Columella** in the first century BC postulated that diseases were caused by invisible beings (***Animalia minuta***) inhaled or ingested.
- **Fracastorius of Verona (1546)** proposed a ***Contagium vivum*** as a possible cause of infections disease and **Von Plenciz (1762)** suggested that each disease was caused by a separate agent.

❖ DISCOVERY OF MICROBES AND THE DAWN OF MICROBIOLOGY

- Microbiology is the **study of living organisms of microscopic size.**
- The term microbiology was given by French chemist **Louis Pasteur (1822-95).**
- Microbiology is said to have its roots in the **great expansion and development** of the biological sciences that took place after **1850.**
- The term microbe was **first used by Sedillot (1878).**

The discovery of microbiology as a discipline could be" traced along the following historical eras :

- Discovery Era
- Transition Period
- Golden Age
- In 20th Century: Era of Molecular Biology

The Discovery Era

- **Robert Hooke,** a **17th-century** English scientist, was the first to use a lens to observe the smallest unit of tissues he called **"cells."** Soon after, the Dutch amateur biologist **Anton van Leeuwenhoek** observed what he called **"animalcules"** with the use of his homemade microscopes.

- **Antonie van Leeuwenhoek (1632-1723)** of **Delft, Holland (Netherland)** was the first person to observe and accurately describe microorganisms **(bacteria and protozoa)** called **'animalcules'** (little animals) in **1676.**
- Actually he was a Dutch linen merchant but spent much of his spare time **constructing simple microscopes composed of double convex lenses held between two silver plates.** He constructed over **250** small powerful microscopes that could magnify around **50-300** times.
- **Leeuwenhoek** was the first person to produce precise and **correct descriptions of bacteria and protozoa** using a **microscope he made himself.**

- Because of this extraordinary contribution to microbiology, **Antonie van Leeuwenhoek is considered as the "Father of microbiology".**
- **Antonie van Leeuwenhoek is also considered to be the father of bacteriology and protozoology (protistology).**
- He wrote over 200 letters which were transmitted as a series of letters from 1674-1723 to Royal Society in London during a 50 years period.

Transition Period

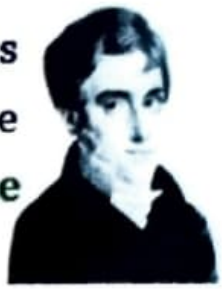
- When microorganisms were known to exist, most scientists believed that such simple life forms could surely arise through **spontaneous generation.**
- That is to say life was thought to spring spontaneously from mud and lakes or anywhere with sufficient nutrients.
- This concept was so compelling that it persisted until late into the **19th century.**
- The main aspects were to solve the controversy over a spontaneous generation which includes experimentation mainly of **Francesco Redi**, John Needham, **Lazzaro Spallanzani** and **Nicolas Appert**, etc, and to know the disease transmission which mainly includes the work of **Ignaz Semmelweis** and **John Snow.**

- **Francesco Redi (1626-1697):** The ancient belief in spontaneous generation was first of all challenged by **Redi**, an **Italian physician**, who carried out a series of experiments on decaying meat and its ability to produce maggots spontaneously.

- **John Needham (1713-1781):** He was probably the greatest supporter of the **theory of spontaneous generation.** He proposed that tiny organisms the animalcules arose spontaneously on his **mutton gravy**. He covered the flasks with cork as done by **Redi** and even heated some flasks. Still the microbes appeared on mutton broth.

- **Lazzaro Spallanzani (1729-1799):** He was an **Italian Naturalist** who attempted to refute Needham's experiment. He **boiled beef broth for longer period**, removed the air from the flask and then sealed the container.

- Followed incubation no growth was observed by him in these flasks. He showed that the heated nutrients could still grow animalcules when exposed to air by simply making a small crack in the neck. **Thus Spallanzani disproved the doctrine of spontaneous generation.**



- **Nicolas Appert** followed the idea of **Spallanzani's work**. He was a **French wine maker** who showed that soups and liquids can be preserved by heating them extensively in **thick champagine bottles.**

- **Ignaz Semmelweis and John Snow** were the two persons who showed a **growing awareness of the mode of disease transmission.**

- **Two German scholars Schulze (1815-1873) and Theodor Schwan (1810-1882)** viewed that air was the **source of microbes** and sought to prove this by passing air through hot glass tubes or strong chemicals into boiled infusions in flasks. The infusion in both the cases remained free from the microbes.

- **George Schroeder and Theodor Von Dusch (1854)** were the first to introduce the idea of **using cotton plugs for plugging microbial culture tubes.**

- **Darwin (1859)** in his book, '**Origin of the Species**' showed that the human body could be conceived as a creature susceptible to the **laws of nature**. He was of the opinion that disease may be a biological phenomenon, rather than any magic.

The Golden Age

- The Golden age of microbiology **began with the work of Louis Pasteur and Robert Koch** who had their own research institute.
- More important there was an acceptance of their work by the scientific community throughout the world and a willingness to continue and expand the work. During this period, we see the **real beginning of microbiology as a discipline of biology.**
- The concept of spontaneous generation was finally put to rest by the French chemist **Louis Pasteur** in an inspired set of experiments involving **a goose necked flask.** When he boiled broth in a flask with a straight neck and left it exposed to air, **organisms grew.**


Louis Pasteur

- When he did this with his goose-necked flask, **nothing grew**. The S-shape of this second flask trapped dust particles from the air, **preventing them from reaching the broth.**
- By showing that he could allow air to get into the flask but not the particles in the air, Pasteur proved that it was the **organisms in the dust that were growing in the broth.**

➢ **Pasteur,** thus in **1858** finally resolved the **controversy of spontaneous generation versus biogenesis** and proved that microorganisms are not spontaneously generated from inanimate matter but arise from other **microorganisms**.

- He also found that **fermentation of fruits and grains**, resulting in alcohol, was brought about by microbes and also determined that **bacteria were responsible for the spoilage of wine during fermentation.**
- Pasteur in **1862** suggested that mild heating at **62.8°C (145°F) for 30 minutes** rather than boiling was enough to destroy the undesirable organisms without ruining the taste of the product, the process was called Pasteurization.

- **Pasteurization** was introduced into the **United States on a commercial basis** in **1892**. His work led to the development of the germ theory of disease.
- Louis Pasteur is known as the **"Father of Modern Microbiology / Father of Bacteriology.**
- **John Tyndall (1820 – 1893):** An English physicist, deal a final blow to spontaneous generation in **1877**. He conducted experiments in an aseptically designed box to prove that **dust indeed carried the germs.**


John Tyndall

- He demonstrated that if no dust was present, sterile broth remained free of microbial growth **for indefinite period even if it was directly exposed to air.**
- He discovered highly resistant bacterial structure, later known as **endospore, in the infusion of hay.**
- Prolonged boiling or intermittent heating was necessary to kill these spores, to make the infusion completely sterilized, **a process known as Tyndallization.**

- Around the same time that Pasteur was doing his experiments, a doctor named **Robert Koch** was working on finding the causes of some very nasty animal diseases **(first anthrax, and then tuberculosis).**
- He gave the **first direct demonstration** of the role of bacteria in causing disease. He was a German physician who first of all isolated anthrax bacillus (***Bacillus anthracis***, the cause of anthrax) in **1876.**
- He perfected the technique of isolating bacteria in pure culture. He also introduced the use of solid culture media in **1881** by using gelatin **as a solidifying agent.** In **1882** he discovered ***Mycobacterium tuberculosis***.
- He **proposed Koch postulate** which were published in **1884** and are the corner stone of the ***germ theory*** of diseases and are still in use today to prove the etiology **(specific cause)** of an infectious disease.
- **Koch's four postulates are:**

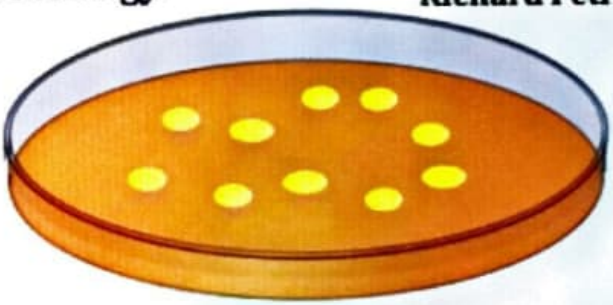
1. The **organism causing the disease** can be **found in sick individuals** but **not in healthy** ones.
2. The organism can be **isolated** and **grown in pure culture**.
3. The organism must cause the disease when it is introduced into a healthy animal.
4. **The organism must be recovered from the infected animal** and shown to be the same as the organism that was introduced.

- The combined efforts of many scientists and most importantly **Louis Pasteur and Robert Koch established the Germ theory of disease.**
- The idea that invisible microorganisms are the cause of disease is **called germ theory.** This was another of the important contributions of Pasteur to microbiology.
- It emerged not only from his experiments disproving spontaneous generation but also from his search for the infectious organism **(typhoid)** that caused the deaths of three of his daughters.

Fanne Eilshemius Hesse

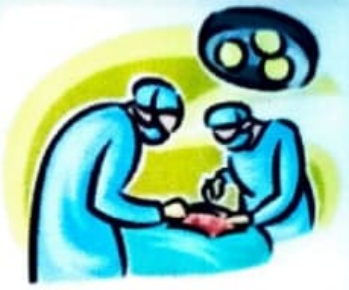
- **Fanne Eilshemius Hesse (1850 – 1934)** one of Koch's assistant first **proposed the use of agar in culture media**. Agar was superior to gelatin because of its higher melting **(i.e. 96°C)** and solidifying **(i.e. 40-45°C)** points than gelatin and was not attacked by most bacteria.
- **Koch's another assistant Richard Petri** in 1887 developed the **Petri dish (plate),** a container used for solid culture media. Thus contribution of **Robert Koch, Fannie Hesse and Richard Petri** made possible the isolation of pure cultures of microorganisms and directly stimulated progress in all areas of microbiology.

Richard Petri



Petri dish (plate)

Development in Medicine and Surgery



- Once scientists knew that microbes caused disease, it was only a matter of time before **medical practices improved dramatically.**
- Surgery used to be as dangerous as not doing anything at all, but once **aseptic (sterile) technique** was introduced, recovery rates improved dramatically.
- Hand washing and quarantine of infected patients reduced the spread of disease and made hospitals into a place to get treatment instead of a place to die.

- **Lord Joseph Lister (1827-1912):** A famous **English surgeon** is known for his notable contribution to the **antiseptic treatment** for the prevention and cure of **wound infections.** Lister concluded that wound infections too were due to microorganisms.
- In **1867**, he developed a system of antiseptic surgery designed to prevent microorganisms from entering wounds by the application of phenol on surgical dressings and at times it was sprayed over the surgical areas.
- He also devised a **method to destroy microorganisms** in the **operation theatre** by spraying a fine mist of carbolic acid into the air, thus **producing an antiseptic environment.**

- Thus Joseph Lister was the first to introduce aseptic techniques for control of microbes by the use of physical and chemical agents which are still in use today. Because of this notable contribution, **Joseph Lister is known as the Father of Antiseptic surgery.**

Development of Vaccines

- **Vaccination** was **discovered before germ theory**, but it wasn't fully understood until the time of **Pasteur**.
- In the late **18th** century, milkmaids who contracted the nonlethal **cowpox sickness from the cows** they were milking were spared in deadly **smallpox outbreaks** that ravaged England periodically.
- The physician **Edward Jenner** used pus from **cowpox** scabs to vaccinate people against **smallpox**.

- **Edward Jenner (1749-1823)** an English physician was the first to prevent small pox. He was impressed by the observation that countryside milk maid who contacted cowpox **(Cowpox is a milder disease caused by a virus closely related to small pox)** while milking were subsequently **immune to small pox.**
- On May **14th , 1796** he proved that inoculating people with pus from cowpox lesions provided **protection against small pox.**
- Jenner in 1798, published his results on 23 successful vaccinators.
- Eventually this process was known as vaccination, based on the latin word **'Vacca' meaning cow**. Thus the use of cow pox virus to protect small pox disease in humans became popular replacing the risky technique of immunizing with actual small pox material.
- Jenner's experimental significance was realized by **Pasteur** who next applied this principle to the **prevention of anthrax and it worked.**
- He called the attenuated cultures vaccines **(Vacca = cow)** and the process as vaccination.
- Encouraged by the successful prevention of **anthrax by vaccination**, Pasteur marched ahead towards the service of humanity by making a **vaccine for hydrophobia or rabies.**
- As with Jenner's vaccination for small pox, principle of the preventive treatment of rabies also worked fully which laid the **foundation of modern immunization programme** against many dreaded diseases like **diphtheria, tetanus, pertussis, polio and measles** etc.

- **Elie Metchnikoff (1845-1916)** proposed the **phagocytic theory of immunity in 1883**. He discovered that some **blood leukocytes, white blood** cells protect against disease by engulfing disease causing bacteria. These cells were **called phagocytes** and the process **phagocytosis**. Thus human blood cells also confer immunity, referred to as **cellular immunity.**

Elie Metchnikoff

Development of Chemotherapeutics, Antitoxins and Antibiotics

- **Emile Roux (1853-1933)** and **Alexandre Yersin,** the two notable French bacteriologists demonstrated the **production of toxin** in filtrates of broth cultures of the **diphtheria organism.**

- **Emil von Behring (1854 -1917) and Shibasaburo Kitasato (1852-1931)** both colleagues of Robert Koch, in **1890** discovered **tetanus (lock jaw) antitoxin.**

- Only about a week after the announcement of the discovery of tetanus antitoxin, **Von Behring** in 1890 reported on **immunization against diphtheria by diphtheria antitoxin.**
- The discovery of toxin-antitoxin relationship was very important to the development of **science of immunology.**

Paul Ehrlich

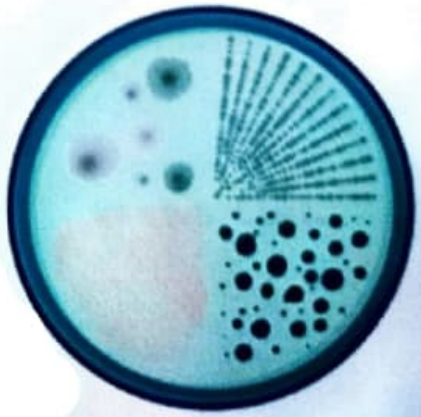
- **Paul Ehrlich (1854-1915)** in 1904 found that the dye **Trypan Red** was active against the trypanosome that causes African sleeping sickness and could be used therapeutically. This dye with antimicrobial activity was referred to as a 'magic bullet'.
- Subsequently in **1910**, Ehrlich in collaboration with **Sakachiro Hata,** a **Japanese physician**, introduced the drug **Salvarsan (arsenobenzol)** as a treatment for syphilis caused by ***Treponema pallidum.***

- **Ehrlich's** work had laid important foundations for many of the developments to come and the use of **Salvarsen** marked the beginning of the eni of **chemotherapy** and the use of chemicals that selectively **inhibit or kill pathogens without causing damage to the patient.**

- **Gerhard Domagk** of Germany in **1935** experimented with numerous synthetic dyes and reported that **Prontosil**, a **red dye** used for staining leather, was active against pathogenic, **Streptococci** and Staphylococci in mice even though it had no effect against that same infectious agent in a test tube.
- In the same year two French scientists **Jacques and Therese Trefonel** showed that the compound **Prontosil** was **broken down** within the body of the animal to **sulfanilamide (Sulfa drug)** the true active factor.
- Domagk was awarded **Nobel prize in 1939** for the discovery of the first **sulpha drug.**
- The credit for the discovery of this first 'wonder drug' **penicillin in 1929** goes to Sir **Alexander Fleming** of England, a Scottish physician and bacteriologist.
- Fleming had been actually interested in searching something that would kill pathogens ever since working on wound infections during the **first world war (1914-1918).**

Alexander Fleming

- **Antibiotics** were discovered completely by accident in the **1920s**, when a solid culture in a **Petri dish** of bacteria was left to sit around longer than usual. As will happen with any food source left sitting around, it became moldy, growing a patch of fuzzy fungus.
- The colonies in the area around the fungal colony were **smaller in size** and seemed to be growing poorly compared to the bacteria on the rest of the plate.

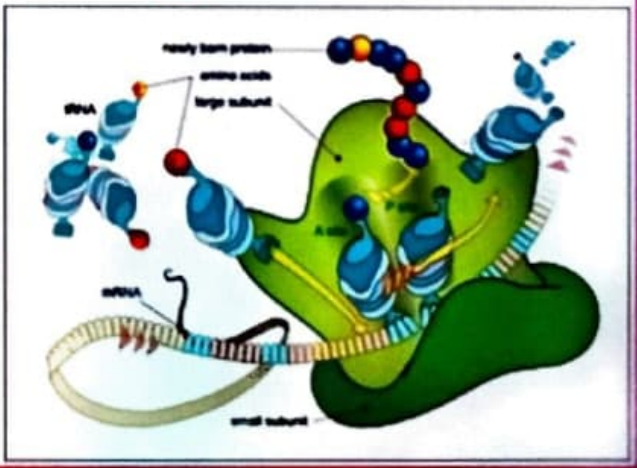


- The compound found to be responsible for this antibacterial action was named **penicillin**. **The first antibiotic**, penicillin was later used to treat people suffering from a variety of bacterial infections and to prevent bacterial infection in burn victims, among many other applications.
- In this way, **Sir Alexander Fleming in 1929 discovered the first antibiotic penicillin.**

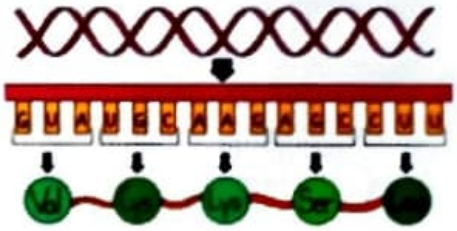
- **Waksman** at the Rutgers university, USA discovered another antibiotic, **streptomycin** produced by two strains of actinomycete, ***Streptomyces griseus*** in 1944.
- **Waksman received the noble prize in 1952** for his discovery of Streptomycin used in the treatment of **tuberculosis**, a bacterial disease caused by ***Mycobacterium tuberculosis*** that had been discovered by **Robert Koch in 1882.**
- By 1950, three other microorganism were identified that produced antibiotics, such as **chloramphenicol (Chloromycetin)** from ***Streptomyces venezuelae*** by **Dr. Paul R. Burkholder** in 1947, **Aureomycin** from ***S. aureofaciens*** by **Dr. B.M. Dugger** in 1948; and **Terramycin** from ***S. rimosus*** by **Finlay, Hobby and collaborators** in 1950.
- A dramatic turn in microbiology research was signaled by the death of **Robert Koch in 1910** and advent of **World war I.**
- The Pasteur Institute was closed, and the German laboratories converted for production of blood components used to treat war infections. **Thus came to an end what many have called the Golden Age of Microbiology**.

➢ In 20th Century: Era of Molecular Biology

- By the **end of 1900,** science of microbiology grew up to the **adolescence stage** and had come to its own as a branch of the **more inclusive field of biology.**



- In the later years the microorganism were picked up as **ideal tools to study various life processes** and thus an **independent discipline** of microbiology, molecular biology was born.
- The relative simplicity of the **microorganism**, their short life span and the genetic homogeneity provided an authentic simulated model to understand the **physiological, biochemical and genetical intricacies** of the living organisms.
- The field of molecular biology made great strides in understanding the **genetic code**, **how DNA is regulated**, and **how RNA is translated into proteins.**



- Until this point, research was focused mainly on plant and animal cells, which are much more complex than bacterial cells.
- When researchers switched to studying these processes in bacteria, many of the secrets of genes and enzymes started to reveal themselves.

❖ Historical Development in the Field of Microbiology

1220-1252	**Rogen Bacon,** disease produced by invisible living creatures.
1546	**Girolamo Fracastoro,** disease was caused by minute 'seed' or 'germ's spread from person to person.
1658	**Athanasius Kircher,** 1st recognize the significance of bacteria and other microbes in disease.
1665	**Robert Hooke,** referred as 'cells'.
1668	**Franceso Redi,** demonstrate the fallacies in the spontaneous generation theory.
1676	**Antony Van Leeuwenhoek** discovers *I* animalcules' .
1688	**Redi Publishes** work on spontaneous generation of maggaot.
1776	**Lazzaro Spallanzani** conducts experiment that dispute spontaneous generation.
1786	**Muller** produces first classification of bacteria.

1798	**Edward Jenner** introduces Cowpox vaccination for small pox.
1799	**Spallanzani** attacks on the theory of spontaneous generation.
1839	**Theodor Schwann (german zoologist)** and Mathias Schleiden **(botanist)** formulate the cell theory.
1857	**Pasteur** shows that lactic acid fermentation is due to a micro-organism.
1858	**Rudolf Virchow,** (all cell originate from pre existing cells).
1861	**Pasteur** shows that microorganism do not arise by **spontaneous generation.**
1867	**Lister** publishes his work on antiseptic surgery.
1869	**Johann Meischer** discovers **nucleic acids.**
1876-77	**Koch** demonstrate that **anthrax** is caused by *Bacillus allthracis.*
1881	**Koch cultures** bacteria on gelatin.
1882	**Koch discovers** tubercle bacillus.
1884	➢ Koch's postulates first published. ➢ Metchnikoff describes phagocytosis. ➢ Autoclave developed. ➢ Gram stain developed.
1885	Pasteur develops rabies vaccine
1887	Petridish developed by Richard Petri.
1892	D. Ivanovski provides evidence for virus causation of T.M.V.
1897	Ross shows that malaria parasite is carried by the mosquito.
1899	Beijerinck proves that a virus particle causes the T.M.V.
1906	August Wasserman develops the first serologic test. for syphilis.
1908	Paul Ehrlich becomes the pioneer of modern chemotherapy to treat syphilis.

1910	**Frances Rous** discovers viruses that can induce cancer.
1915-17	**F. D. Herelle and F. Twort** independently discover bacterial viruses.
1923	First edition of **Bergey's manual.**
1928	**Griffith** discovers bacterial transformation.
1929	**Alexander Fleming** discovers penicillin
1935	**Stanley** crystallizes the T.M.V.
1944	**Avery** shows that DNA carries information during transformation. **Selman Waksman** discovers streptomycin.
1946	**Lederberg and Tatum** describe bacterial conjugation.
1952	**Hershey and Chase** show that bacteriophage inject DNA into host cells. Zinder & Lederberg discover generalized transduction.
1953	**Watson & Crick** propose the double helix structure for DNA.
1954	**Jonas Salk** develops the first polio vaccine.
1957	**Isaacs and Lindenmann** discover the natural antiviral substance, Interferon.
1958	**Lederberg** makes discoveries concerning genetic recombination and the organization of the genetic material of bacteria.
1959	**Korenberg & Ochoa** awarded Nobel prize for the discovery of enzyme which produces artificial DNA and RNA.
1966	**Rous** discovered tumor inducing viruses.
1971	**T.O. Diemer** identifies viroids.
1975	**Kohler & Milstein** develop technique for the production of monoclonal antibodies.
1977	**Recognition of archaeobacteria** as distinct microbial group.
1979	**Henle** identified first virus regularly associated with human cancer and insulin synthesized using rDNA techniques.
1982	Recombinant **Hepatitis B vaccine** developed.

1982-83	**Cech and Altman** discovered catalytic RNA.
1983-84	**Gallo and Montagnier** isolated and identified HIV virus and PCR chain reaction developed by Mullis.
1990	First human **gene therapy testing begun.**
1995	**Lewis, Nusslein and Wieschans** for the study of physiology of genetics of microbes.
1997	**Prussiner** discovery of prions.

❑ SCOPE OF MICROBIOLOGY

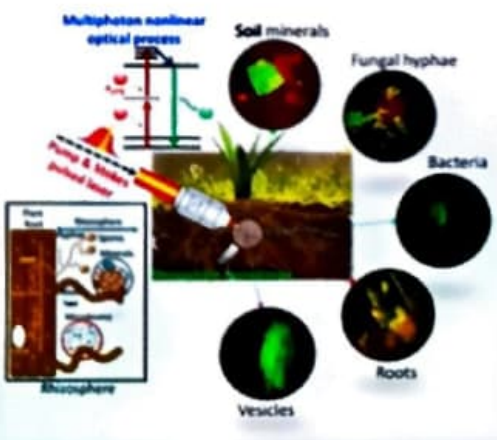
Microbiologists are currently in high demand across a variety of industries and fields all over the world. They are not restricted to only one of them, here are the most sought-after fields and areas in which the importance of microbiology is evidently large:

- ✓ **Food Microbiology**
- ✓ **Environmental Science**
- ✓ **Healthcare and Medicine**
- ✓ **Genetic Engineering**
- ✓ **Biotechnology for Agro-chemistry**
- ✓ **Boards for pollution control**
- ✓ **Biorefineries**
- ✓ **Hospitals**
- ✓ **Universities**
- ✓ **Research Centres**
- ✓ **Forensic Labs**

- Food industry microorganisms are utilized in the preparation of different food products like **cheese, pickles** and **alcohol, bread, vinegar and green olives.**

Environmental Science

- The field of microbiology the field is extensive. From understanding and **applying microbes to understanding and using microbes and bioremediation,** to the control of pests, microbiologists are able to tackle a variety of problems that are prevalent in this area.



➢ Healthcare Sector

- Bacteria and other microbes are **utilized to produce diverse Antibiotics and synthesize vitamins** , which is vital to our bodies. They also are **used in gene therapy** to treat genetic disorders. This is the reason the field of microbiology in this area is growing.

➢ Genetic Engineering

- The field of microbiology within the field is huge due to the rising popularity of the discipline. The microbes' genes can be altered to make beneficial and valuable substances like hormones, enzymes and more.

❖ CAREERS IN MICROBIOLOGY

- With the growing awareness of the value of Microbiology numerous people are rushing to this field to study one of the lucrative courses that are available after the **12th science**. These are the top jobs in Microbiology:

➢ Biotechnologist

- In order to develop and develop items that are able to be utilized in numerous applications Biotechnologists conduct an extensive study of the chemical, physical and genetic properties of microbes.

- They also create easy-to-use products. From pharmaceutics and agriculture to food science and genetics the field of microbiology when working as biotechnologist can be broad.

➢ Food Microbiologist

- When discussing the scope of microbiology food microbiologist is one profile that is highly sought-after. They work to reduce foodborne illness by conducting extensive studies on the microbes that cause disease and their environment, the packaging of food items, food poisoning, laws and regulations, etc.

Medicinal Chemist

- To identify ways to **develop, design and optimize the effectiveness of drugs** made from chemical compounds medical chemists play a important role in the field of **pharmaceuticals**.
- As a **Medical Chemist** is a broad field of microbiology does not just limit itself to the development of **new formulations for drugs** and also involves the **creation of new methods by which drugs are made.**
- If you are looking to pursue the foundation for a Career in Biochemistry it could be the **ideal job.**

Pharmacologist

- As we have discussed, the application of microbiology isn't limited to a few particular fields, but **can be utilized across other industries as well.**
- A pharmacologist is a career where **microbiologists are highly sought-after**.
- Finding and studying the **connections between living and non-living substances** in order to create new medicines is the major task of these experts.

Nanotechnologist

- There are applications for **nanotechnology in nearly every field**, nanotechnology courses comprise elements of biology, chemistry, physics and pharmacology, microbiology etc.

Technical Brewer

- In the industry of beer production technical brewers are the **highest-ranking professionals** who, with their technical and managerial skills supervise, control and manage the equipment and process of brewing.
- These experts must be proficient in terms related to microbiology, biochemistry, and so on.

Marine Biologist

- Marine biologists are a person who is interested in **marine life** and can **identify the factors that disrupt the same.**
- With a variety of microorganisms, **such as algae, fungi, and bacteria** being an integral element of marine life biologists study the physiological and behavioral functions for various species of the marine. They may also study **taxonomy and fossil microbiology.**

Clinical Scientist

- Employed in clinics, hospitals as well as laboratories as well as **research institutions, scientists** try to make use of their expertise in the **fields of medicine and biomedical research.**
- They aid in the development of living organisms and also develop new treatments and medicines for it. For more information, check out our comprehensive guide on how to become Scientist!

Biomedical Scientist

- Typically working in the labs Biomedical scientists work with healthcare professionals like pharmacists or doctors to detect and treat various illnesses by **analyzing different biopsies,** fluids, and other types of samples.

Forensic Scientist

- The field of microbiology can also be apparent when it comes to Forensic Sciences. These experts utilize scientific and analytical expertise and techniques to analyze the **evidence of crime scene** and create legal statements for courts. They are usually involved in laboratory analysis or investigation of crime scenes.

❑ APPLICATIONS OF MICROBIOLOGY

- ✓ **Microbiology** is among the most extensive and complex of biological sciences since it covers a variety of biological disciplines.
- ✓ Apart from investigating the microbiology of microbes it also examines every aspect of human-microbe and environmental interactions. These interactions encompass biology, **genetics, metabolism and disease**, as well as infection, **immunology, chemotherapy as well as industry, genetic engineering and agriculture.**

➢ Medicine

- ✓ The ability to cause disease in certain microbes like.
- ✓ **The Small Pox (Variola virus)**
- ✓ **Cholera (Vibrio cholera)**
- ✓ **Malaria (Plasmodium, protozoa)** etc.
- ✓ They also provide us with the means to their control through antibiotics, as well as other medically significant medications.

➢ Biotechnology

- ✓ Commercial applications comprise the production of **acetone, organic acids, alcohols, enzymes, and a variety of other drugs.**
- ✓ Genetic engineering is the process by which bacteria create important therapeutic substances like **insulin** and human **growth hormone** and **interferon**.

➢ Food

- ✓ **Microorganisms have been utilized to make food items**, starting with wine and brewing through **cheese production, bread making, up to the manufacture Soy Sauce.**
- ✓ Microbes also contribute to food spoilage.

➢ Research

- ✓ Due to their simplicity, they are simpler to study biological processes in **monocellular organisms, compared to multicellular organisms that are complex.**
- ✓ Many copies of a single cell are produced in huge numbers, in a **short time and at a low cost**. This can provide lots of **homogenous material for experiments.**
- ✓ Since they reproduce extremely quickly, they can be useful in studies that involve transfers of **information genetically.**

➢ The environment

- ✓ Microbes play a role in the **cycle of nitrogen, carbon** and as well as **phosphorus (geochemical cycles)**
- ✓ Help to maintain the balance of nature on Earth
- ✓ They can be found in associations with plants in symbiotic relations that **help to maintain soil fertility**. They could also be used to rid the **earth of toxic substances (bio-remediation).**

➢ Future of Microbiology

- ✓ **Future challenges** will include coming up with **new ways to fight diseases**, cut pollution and feed the **world's growing population.**
- ✓ **AIDS hemorrhagic fevers**, hemorrhagic fevers, and other infections
- ✓ **Develop new medicines, vaccines and vaccines**. Utilize the methods of molecular biology or rDNA to address the issues
- ✓ **Host-pathogen relationships**
- ✓ Examine the **role of microorganisms** in the subject.
- ✓ Food sources of top quality as well as other useful products like **enzymes for industrial applications**
- ✓ Degrade toxic and **harmful wastes** and **pollutants**
- ✓ As vectors for **treating ailments and increase the productivity of agricultural crops.**

UNIT- I
(CHAPTER- 2)
PROKARYOTES & EUKARYOTES

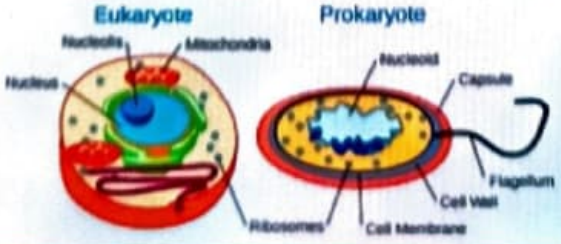
Points to be covered in this topic

1. PROKARYOTIC CELL
2. CHARACTERISTICS OF PROKARYOTIC CELL
3. PROKARYOTIC CELL STRUCTURE
4. EUKARYOTIC CELL
5. STRUCTURE OF EUKARYOTIC CELL
6. DIFFERENCE BETWEEN EUKARYOTIC CELL & PROKARYOTIC CELL

❑ PROKARYOTIC CELL

Prokaryotic cells are **single-celled microorganisms** known to be the earliest on earth. Prokaryotes include **Bacteria and Archaea.**

- The photosynthetic prokaryotes include **cyanobacteria** that perform **photosynthesis.**
- A prokaryotic cell consists of a **single membrane** and therefore, all the reactions occur within the **cytoplasm.** They can be **free-living or parasites.**



➢ Characteristics of Prokaryotic Cell

a. Prokaryotic cells have different characteristic features. The characteristics of the prokaryotic cells are mentioned below.

b. They lack a **nuclear membrane.**

c. **Mitochondria, Golgi bodies, chloroplast, and lysosomes** are **absent**.

d. The **genetic material** is present on **a single chromosome**.

e. The **histone proteins**, the important constituents of eukaryotic chromosomes, are lacking in them.

f. The cell wall is made up of **carbohydrates and amino acids**.

g. The **plasma membrane** acts as the **mitochondrial membrane** carrying **respiratory enzymes.**

h. They divide asexually by **binary fission**. The sexual mode of **reproduction involves conjugation**.

❖ Prokaryotic Cell Structure

A prokaryotic cell does **not have a nuclear membrane**. However, the genetic material is present in a region in the cytoplasm known as the **nucleoid**. They may be **spherical, rod-shaped, or spiral**. A prokaryotic cell structure is as follows:

➢ **Capsule**– It is an outer protective covering found in the bacterial cells, in addition to the cell wall.

- It helps in moisture retention, protects the cell when engulfed, and helps in the attachment of cells to nutrients and surfaces.

➢ **Cell Wall**– It is the outermost layer of the cell which gives shape to the cell.

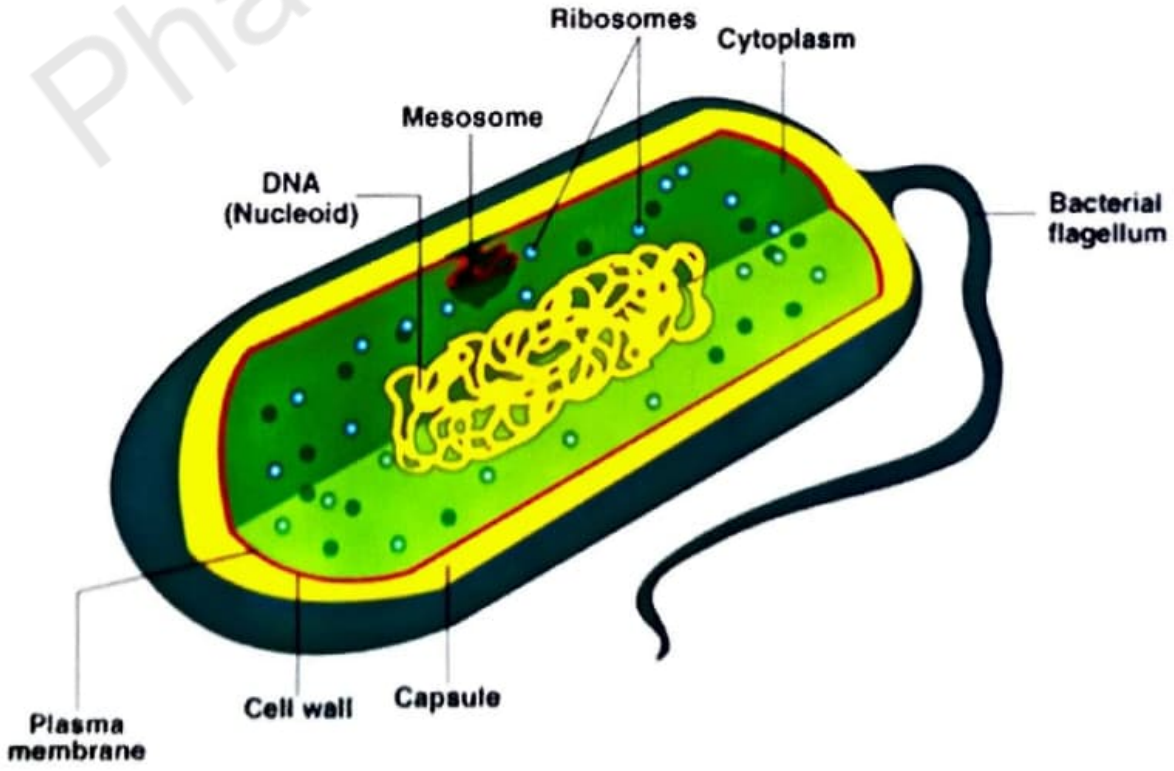
➢ **Cytoplasm**– The cytoplasm is mainly composed of **enzymes, salts, cell organelles and is a gel-like component.**

➢ **Cell Membrane**– This layer surrounds the cytoplasm and regulates the entry and exit of substances in the cells.

- **Pili–** These are hair-like outgrowths that attach to the surface of other bacterial cells.
- **Flagella–** These are long structures in the form of a whip, that help in the locomotion of a cell.
- **Ribosomes–** These are involved in protein synthesis.
- **Plasmids–** Plasmids are non-chromosomal **DNA structures**. These are not involved in reproduction.
- **Nucleoid Region–** It is the region in the cytoplasm **where the genetic material is present.**
- A prokaryotic cell lacks certain organelles like mitochondria, endoplasmic reticulum, and Golgi bodies.

❖ Prokaryotic Cell Diagram

The prokaryotic cell diagram given below represents a **bacterial cell**. It depicts the absence of a true nucleus and the presence of a flagellum that differentiates it from a eukaryotic cell.



Components of Prokaryotic Cells

The prokaryotic cells have four main components:

- **Plasma Membrane-** It is an outer protective covering of phospholipid molecules which separates the cell from the surrounding environment.
- **Cytoplasm-** It is a **jelly-like substance** present inside the cell. All the cell organelles are suspended in it.
- **DNA-** It is the genetic material of the cell. All the **prokaryotes possess a circular DNA.** It directs what proteins the cell creates. It also regulates the actions of the cell.
- **Ribosomes-** Protein synthesis occurs here.

Some prokaryotic cells possess cilia and flagella which helps in locomotion.

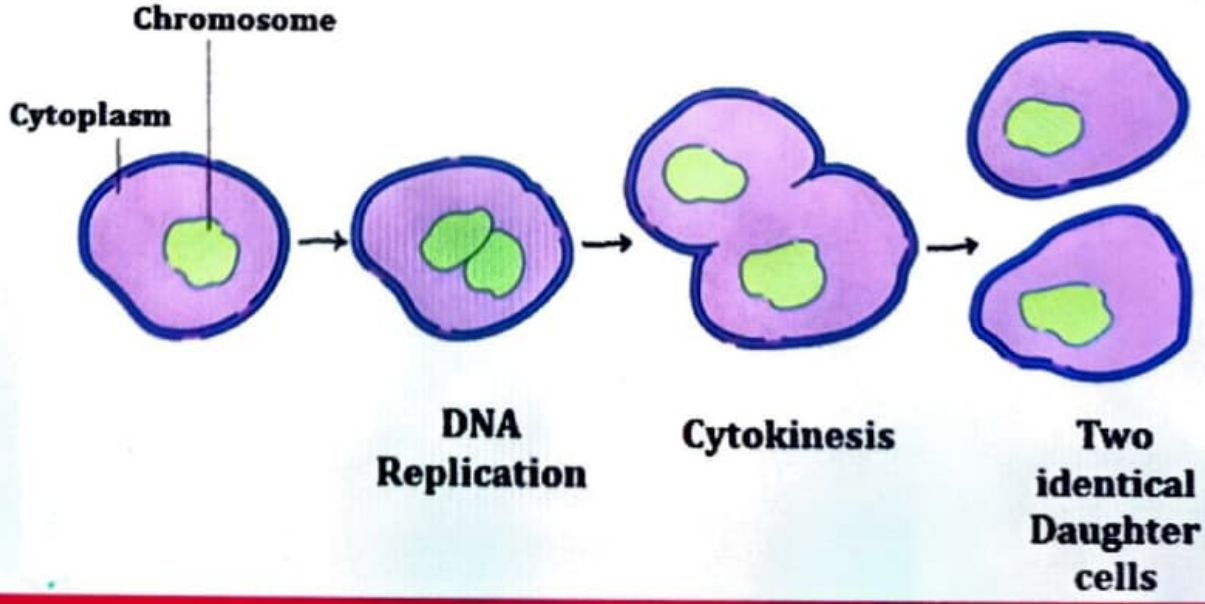
❖ Reproduction in Prokaryotes

A prokaryote reproduces in two ways:

- **Asexually by binary fission**
- **Sexually by conjugation**

Binary Fission

1. The DNA of an organism replicates and the new copies attach to the cell membrane.
2. The cell wall starts **increasing in size** and starts moving inwards.
3. A cell wall is then formed between each **DNA**, **dividing the cell into two daughter cells.**



➢ Recombination

In this process, genes from one bacteria are transferred to the genome of other bacteria. It takes place in three ways- **conjugation, transformation, transduction.**

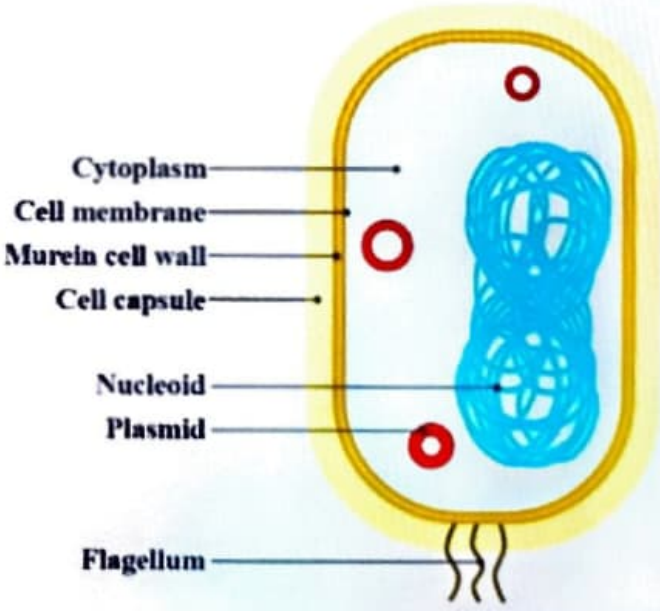
- **Conjugation** is the process in which genes are transferred between two bacteria through a protein tube structure called a pilus.
- **Transformation** is the mode of sexual reproduction in which the DNA from the surroundings is taken by the bacterial cell and incorporated in its DNA.
- **Transduction** is the process in which the genetic material is transferred into the bacterial cell with the help of viruses. Bacteriophages are the virus that initiates the process.

✓ Examples of Prokaryotic Cells

The examples of the prokaryotic cells are mentioned below:

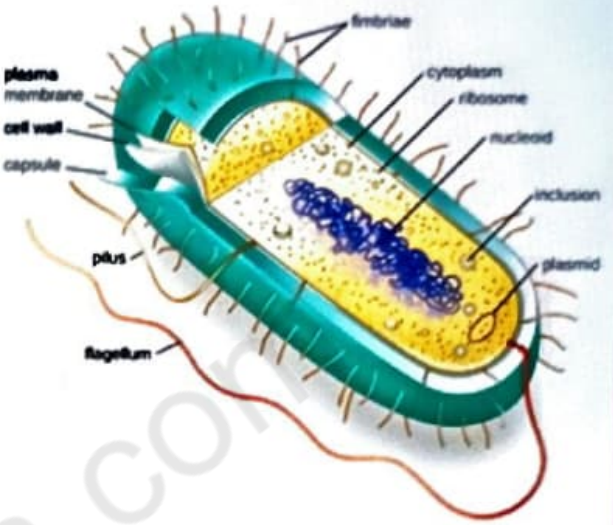
❖ Bacterial Cells

- These are **unicellular organisms** found everywhere on earth from soil to the human body.
- They have different **shapes and structures.**
- The cell wall is composed of **peptidoglycan** that provides **structure to the cell wall.**
- Bacteria have some unique structures such as **pili, flagella and capsule.**
- They also possess **extra chromosomal DNA known as plasmids.**
- They have the ability to form tough, dormant structures known as endospores that helps them to **survive under unfavorable conditions.**
- The endospores become active when the **conditions are favourable again.**



➢ Archaeal Cells

- **Archaebacteria** are unicellular **organisms similar to bacteria in shape and size.**
- They are found in **extreme** environments **such as hot springs and other places such as soil, marshes, and even inside humans.**
- They have a cell wall and flagella. **The cell wall of archaea does not contain peptidoglycan.**
- The membranes **of the archaea have different lipids** with a completely **different stereochemistry.**
- Just like **bacteria, archaea have one circular chromosome.** They also **possess plasmids.**



❑ EUKARYOTIC CELL

- **Eukaryotic cells** have a nucleus enclosed within the nuclear membrane and form large and complex organisms.
- **Protozoa, fungi, plants, and animals** all have eukaryotic cells. They are classified under the **kingdom Eukaryota.**
- They can maintain different environments in a single cell that allows them to **carry out various metabolic reactions.**
- This helps them grow many times larger than the prokaryotic cells.

➢ Characteristics of Eukaryotic Cells

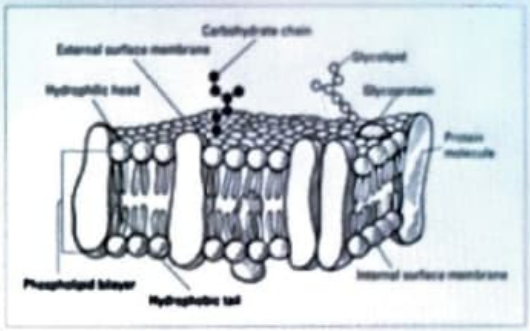
The features of eukaryotic cells are as follows:

1. Eukaryotic cells have the nucleus enclosed **within the nuclear membrane.**
2. The cell has **mitochondria.**
3. **Flagella and cilia are the locomotory organs in a eukaryotic cell.**
4. A **cell wall** is the outermost layer of the **eukaryotic cells.**
5. The cells divide by a process called **mitosis.**
6. The eukaryotic cells contain a **cytoskeletal structure.**
7. The **nucleus contains a single, linear DNA,** which carries all the genetic information.

❑ STRUCTURE OF EUKARYOTIC CELL

The eukaryotic cell structure comprises the following:

➢ Plasma Membrane



- The plasma membrane **separates the cell from the outside environment.**
- It comprises specific embedded proteins, which help in the exchange of substances in and out of the cell.

➢ Cell Wall

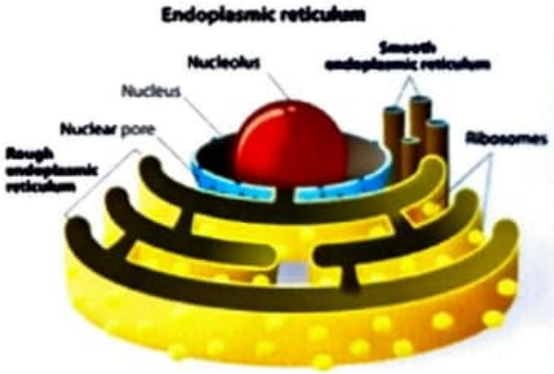
- A cell wall is a **rigid structure present outside** the plant cell. It is, **however**, absent in animal cells.
- It provides shape to the cell and helps in **cell-to-cell interaction.**
- It is a **protective layer** that **protects the cell** from any injury or pathogen attacks.
- It is composed of **cellulose, hemicellulose, pectins, proteins**, etc.

➢ Cytoskeleton

- The cytoskeleton is present **inside the cytoplasm**, which consists of **microfilaments, microtubules, and fibres** to provide **perfect shape to the cell,** anchor the organelles, and stimulate the cell movement.

➢ Endoplasmic Reticulum

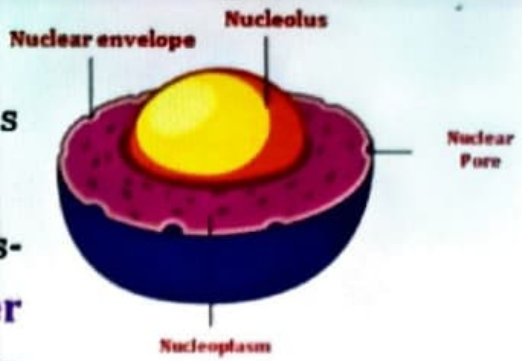
It is a network of small, tubular structures that divides the cell surface into two parts: luminal and extra luminal.



Endoplasmic Reticulum is of two types:

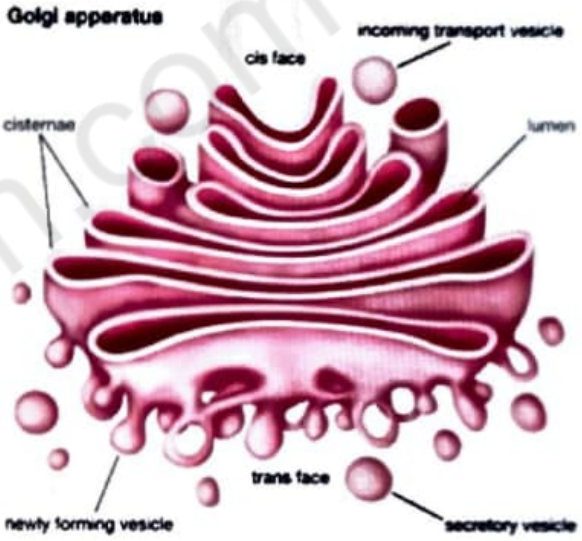
- **Rough Endoplasmic Reticulum** contains **ribosomes.**
- **Smooth Endoplasmic Reticulum** that lacks ribosomes and is therefore smooth.

Nucleus



- The nucleoplasm enclosed within the nucleus **contains DNA and proteins.**
- The nuclear envelop consists of two layers- the **outer membrane and the inner membrane.** Both the membranes are **permeable to ions, molecules, and RNA material.**
- Ribosome production also takes place inside the nucleus.

Golgi Apparatus



- It is **made up of flat disc-shaped** structures called **cisternae.**
- It is absent in red blood **cells of humans and sieve cells of plants.**
- They are arranged **parallel and concentrically near the nucleus.**
- It is an important site for the formation of **glycoproteins and glycolipids.**

Ribosomes

- These are the **main site for protein synthesis** and are composed of proteins and ribonucleic acids.

Mitochondria

- These are also known as **"powerhouse of cells"** because they produce energy.
- It consists of an outer membrane and an inner membrane. The inner membrane is **divided into folds** called **cristae.**
- They help in the regulation of cell metabolism.

Lysosomes

- They are known as **"suicidal bags"** because they possess hydrolytic enzymes to digest protein, lipids, carbohydrates, and nucleic acids.

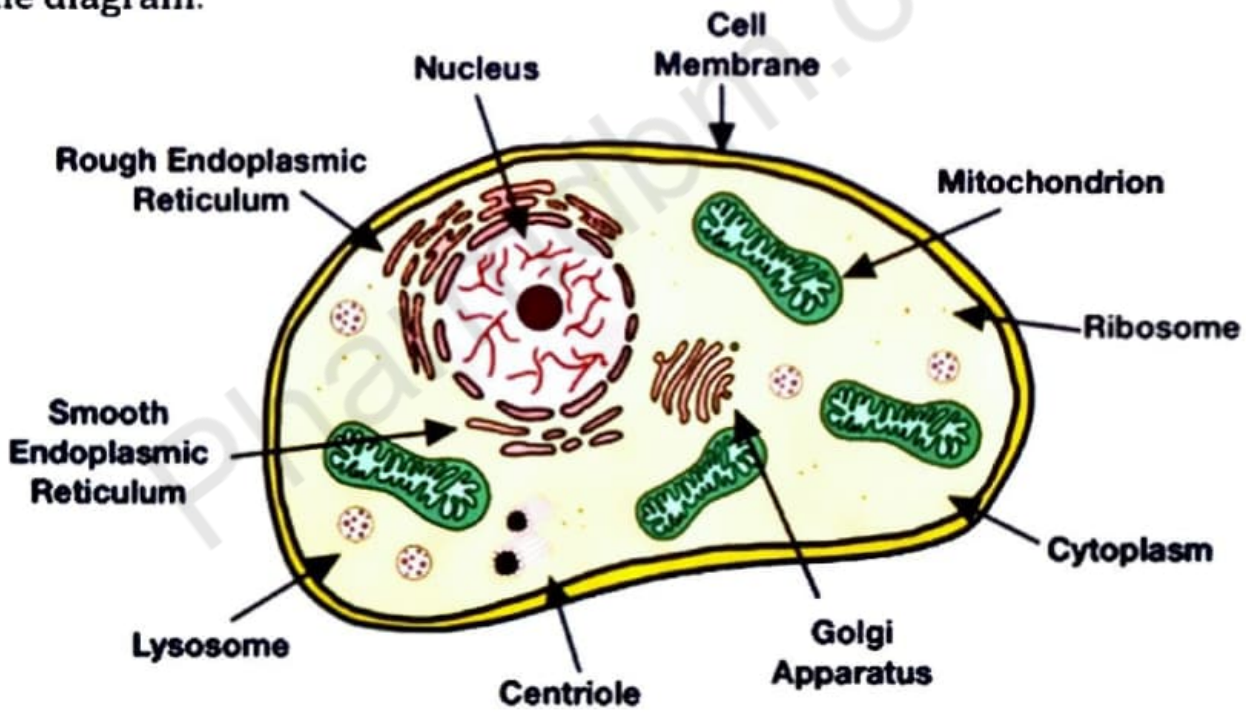
➢ Plastids

These are double-membraned structures and are found only in **plant cells**. **These are of three types:**

- **Chloroplast** that contains chlorophyll and is involved in photosynthesis.
- **Chromoplast** that contains a pigment called carotene that provides the plants yellow, red, or orange colours.
- **Leucoplasts** that are colourless and store oil, fats, carbohydrates, or proteins.

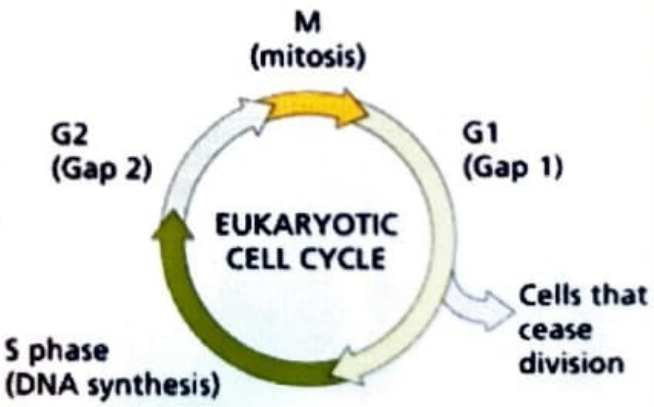
➢ EUKARYOTIC CELL DIAGRAM

- Eukaryotic cell diagram mentioned below depicts different cell organelles **present in eukaryotic cells.** The nucleus, endoplasmic reticulum, cytoplasm, mitochondria, ribosomes, lysosomes are clearly mentioned in the diagram.



❖ Eukaryotic Cell Cycle

The eukaryotic cells divide during the **cell cycle.** The cell passes through **different stages** during the cycle. There are various checkpoints between each stage.



➢ Quiescence (G0)

- This is known as the **resting phase**, and the **cell does not divide** during this stage. The cell cycle starts at this stage. The cells of the liver, kidney, neurons, and stomach all reach this stage and can **remain there for longer periods**. Many cells do not enter this stage and divide indefinitely throughout their lives.

➢ Interphase

- In this stage, the cells grow and take in nutrients to prepare them for the division. **It consists of three checkpoints:**
- **Gap 1 (G1)** – Here the cell enlarges. The proteins also increase.
- **Synthesis (S)** – DNA replication takes place in this phase.
- **Gap 2 (G2)** – The cells enlarge further to undergo mitotic division.

➢ Mitosis

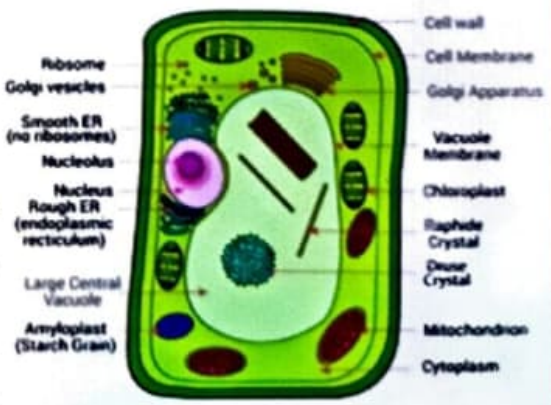
Mitosis involves the following stages:

- ✓ Prophase
- ✓ Prometaphase
- ✓ Metaphase
- ✓ Anaphase
- ✓ Telophase
- ✓ Cytokinesis

➢ On division, each daughter cell is an exact replica of the original cell.

Examples of Eukaryotic Cells

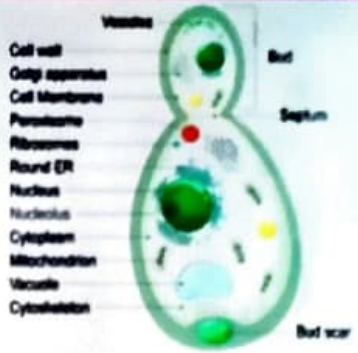
Eukaryotic cells are exclusively found in **plants, animals, fungi, protozoa, and other complex organisms.** The examples of eukaryotic cells are mentioned below:



➢ Plant Cells

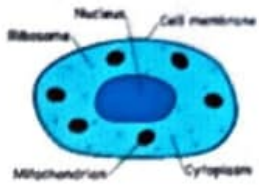
- The cell wall is made up of cellulose, which provides support to the plant. It has a large vacuole which maintains the turgor pressure.
- The plant cell contains chloroplast, which aids in the process of photosynthesis.

➢ Fungal Cells



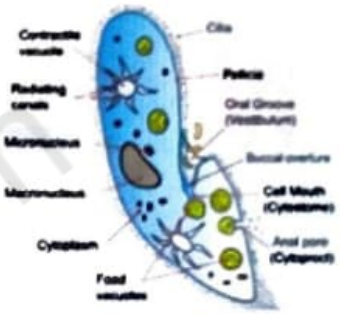
The cell wall is made of **chitin**. Some fungi have holes known as septa which allow the **organelles and cytoplasm to pass through them.**

➢ Animal Cells



These do not have cell walls. Instead, **they have a cell membrane**. That is why animals have varied shapes. They have the ability to perform **phagocytosis and pinocytosis.**

➢ Protozoa



Protozoans are **unicellular organisms**. Some protozoa have **cilia for locomotion**. A thin layer called **pellicle provides supports to the cell.**

❖ DIFFERENCE BETWEEN PROKARYOTIC AND EUKARYOTIC CELLS

Though these two classes of cells are quite different, they do possess some common characteristics. For instance, both possess cell membranes and ribosomes, but the similarities end there. The complete list of differences between prokaryotic and eukaryotic cells is summarized as follows:

➢ Prokaryotic Cell

- The term **"prokaryote"** is derived from the Greek word ***"pro"***, (meaning: before) and ***"karyon"*** **(meaning: kernel).** It translates to ***"before nuclei."***
- Prokaryotes are one of the **most ancient groups** of living organisms on earth, with fossil records dating back to almost **3.5 billion years ago.**
- These prokaryotes thrived in the **earth's ancient environment,** some using up chemical energy and others using the sun's energy.
- These extremophiles thrived for millions of years, **evolving and adapting. Scientists speculate that these organisms gave rise to the eukaryotes.**
- Prokaryotic cells are comparatively **smaller and much simpler than eukaryotic cells.** The other defining characteristic of prokaryotic cells is that it does not possess membrane-bound cell organelles such as a nucleus. **Reproduction happens through the process of binary fission.**

➢ Eukaryotic Cell

- The term "**Eukaryotes**" is derived from the Greek word "*eu*", (meaning: good) and "***karyon***" (meaning: kernel), therefore, translating to "***good or true nuclei.***"
- Eukaryotes are **more complex and much larger than prokaryotes**. They include almost all the major kingdoms except **kingdom monera.**
- Structurally, **eukaryotes possess a cell wall,** which supports and protects the **plasma membrane.**
- The cell is surrounded by the **plasma membrane** and it controls the **entry and exit of certain substances.**
- The **nucleus contains DNA**, which is responsible for storing all genetic information. The nucleus is **surrounded by the nuclear membrane.**
- Within the nucleus exists the nucleolus, and it plays a **crucial role in synthesizing proteins.**
- Eukaryotic cells also contain **mitochondria**, which are responsible for the creation of **energy, which is then utilized by the cell.**

	PROKARYOTES	EUKARYOTES
Type of Cell	Always unicellular	Unicellular and multi-cellular
Cell size	Ranges in size from 0.2 µm – 2.0 µm in diameter	Size ranges from 10 µm – 100 µm in diameter
Cell wall	Usually present; chemically complex in nature	When present, chemically simple in nature
Nucleus	Absent. Instead, they have a nucleoid region in the cell	Present
Ribosomes	Present. Smaller in size and spherical in shape	Present. Comparatively larger in size and linear in shape
DNA arrangement	Circular	Linear

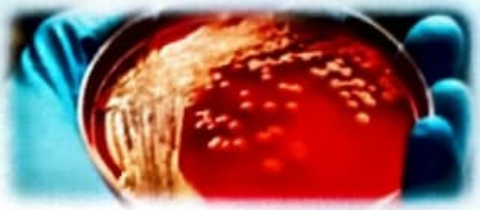
Mitochondria	Absent	Present
Cytoplasm	Present, but cell organelles absent	Present, cell organelles present
Endoplasmic reticulum	Absent	Present
Plasmids	Present	Very rarely found in eukaryotes
Ribosome	Small ribosomes	Large ribosomes
Lysosome	Lysosomes and centrosomes are absent	Lysosomes and centrosomes are present
Cell division	Through binary fission	Through mitosis
Flagella	The flagella are smaller in size	The flagella are larger in size
Reproduction	Asexual	Both asexual and sexual
Example	Bacteria and Archaea	Plant and Animal cell

UNIT- I
(CHAPTER- 3)
CULTURE MEDIA

Points to be covered in this topic

1. INTRODUCTION
2. COMMON INGREDIENTS OF CULTURE MEDIA
3. TYPES OF CULTURE MEDIA BASED ON CONSISTENCY/ PHYSICAL STATE
4. TYPES OF CULTURE MEDIA BASED ON OXYGEN REQUIREMENT
5. TYPES OF CULTURE MEDIA BASED ON CHEMICAL COMPOSITION/APPLICATION
6. TYPES OF SPECIAL PURPOSE CULTURE MEDIA
7. ROLE OF FERMENTATION MEDIA
8. APPLICATION OF CULTURE MEDIA

❑ INTRODUCTION



- **Culture media** are mediums that **provide essential nutrients** and **minerals** to support the **growth of microorganisms** in the laboratory.
- **Microorganisms** have varying nature, **characteristics, habitat,** and **even nutritional requirements,** thus it is impossible to culture them with one type of culture media.
- **However**, there are also microorganisms that can't grow on a culture media at all in any condition – **these are called obligate parasites.**

Common ingredients of culture media

- **Peptone**- Source of carbon and nitrogen.
- **Beef extract**- Source of amino acid, vitamins, minerals.
- **Yeast extract**- Source of vitamin, carbon, nitrogen.
- **Distilled water**
- **Agar**- Solidifying agent.

What is a Defined medium?

- A defined medium has a **known quantity of all ingredients**, like carbon source **(Glucose or Glycerol)** and nitrogen source (Ammonium salt or Nitrate as inorganic nitrogen).
- The medium needs in metabolic, nutritional, and physiological growth experiments. **(Czapek Dox Medium)**

What is an Undefined medium?

- This medium has **different complex ingredients** in unknown quantities, for example- yeast extract, beef, various salts, and enzymatic protein. (Potato dextrose agar, MacConkey agar)

What is Complex media?

- This media **is other than basal media**; it has added ingredients to bring the characteristics of microorganisms with **unique nutrients.**

❖ Types of culture media based on consistency/ physical state

1. Solid medium **2. Semi-solid medium**

3. Liquid medium

1. Solid media

➢ Principle of Solid Media

- It is for the isolation of bacteria as a **pure culture on a solid medium.**
- Robert Koch realized the use of **solid media.**
- Agar is used to hardening the media at **1.5- 2.0%** concentration. Solid media allows the growth of bacteria as colonies by streaking on the medium. It solidified at **37 degrees Celsius.**
- Agar is an **un-branched polysaccharide** extracted from **red algae species like Gelidium. Colonies identification** is done on this medium.

Examples of Solid Media

- ✓ Nutrient agar, MacConkey agar, Blood agar, Chocolate agar.
- ✓ Growth of bacteria on solid medium appear as smooth, rough, mucoid, round, irregular, filamentous, punctiform.

2. Semi-solid media

➢ Principle of Semi-solid media

- This media shows the motility of bacteria and the cultivation of **micro-aerophilic bacteria.** This media has agar at a concentration of 0.5% or less. It has a jelly consistency.
- **Examples of Semi-solid media**
- **Stuart's and Amies media,** Hugh and Leifson's oxidation fermentation medium and Mannitol motility media.
- The growth of bacteria in semi-solid appears as a thick line in the medium.

3. Liquid media

➢ **Principle of Liquid media**

- This media shows the **growth of a large number of bacteria.**
- It is called Broth that allows bacteria to grow **uniformly with turbidity.** The growth occurs at **37°C** in an **incubator for 24hrs.**
- Liquid media **don't have the addition of agar;** it is for **fermentation studies.**

❖ **Examples of Liquid media**

✓ Nutrient broth, Tryptic soy broth, MR-VP broth, phenol red carbohydrate broth.

✓ Growth of bacteria in liquid media- Turbidity is seen at the end of the broth.

❖ Types of culture media based on oxygen requirement

- Microorganisms have different requirements for growth depending on oxygen requirements.

1. Aerobic media

- In this media, it is easy to cultivate microbes, on solid media, the growth occurs by keeping the culture in the incubator.
- It shows the growth; of non-fastidious microorganisms.

Examples of aerobic media are- liquid media, solid media

Peptone water- 1%peptone + 0.5% Nacl +100ml water.

Nutrient agar- Nutrient broth +2% agar.

2. Anaerobic media

- The media cultivates anaerobic bacteria at **low oxygen, reducing oxidation-reduction potential.**
- Anaerobic media contains extra nutrients like **vitamin K, hemin and oxygen** that get reduced by a physical or chemical process.
- The addition of **glucose (1%), thioglycolate (0.1%), ascorbic acid (0.1%), cysteine (0.05%),** or iron fillings added to cause the medium to reduce.
- The medium is **boiled in a water bath** to force out **dissolved oxygen and packed with sterile paraffin.**

Examples of Anaerobic media

- RCM (Robertson cooked meat) isolation for *Clostridium* sp.
- **Thioglycolate broth**– It has sodium glycolate that maintains low oxygen.

❖ Types of culture media based on chemical composition/application

➢ There are seven routine laboratory media.

1. **Basal media**
2. **Enriched media**
3. **Selective media**
4. **Enrichment media**
5. **Indicator media or differential media**
6. **Transport media**
7. **Storage media**

1. Basal media

- This media is simple as it **enhances the growth** of many microorganisms. It's a **routinely used medium in the lab, having Carbon and Nitrogen.**
- This media allows the growth; of non - fastidious bacteria without any enrichment source; used for sub-culturing. It's a **non-selective medium.**
- ***Staphylococcus*** and ***Enterobacteriaceae*** grow in this media.
- **Examples of Basal media**
- Nutrient Agar, Peptone water.

2. Enriched media

- This media requires the **addition of other substances like blood, egg, or serum.**
- An enriched media allows the growth of devised microorganisms but **inhibits other and fastidious microbes grow** as they require nutrients like vitamins and growth-promoting substances.

Example of Enriched media

- Blood agar, Chocolate agar, LSS, Monsur's taurocholate, Lowenstein Jensen media. Blood agar identifies hemolytic bacteria, chocolate media for *N. gonorrhea.*

3. Selective media

- As by name, we can tell, **this media shows the growth of selective**; microbes or desired microorganisms and **inhibits the growth of unwanted microbes.**
- The inhibition occurs by adding bile salts, antibiotics, dyes, PH adjustments.
- Media is agar-based; any media is possible to transform into selective by adding inhibitory agar.

❖ Examples of Selective media

S.N.	Culture media	Inhibiting substances	Bacteria
1	**Thayer Martin Agar**	Contains antibiotics; vancomycin, Colistin, and Nystatin	Used for Neisseria gonorrhoeae
2	**Mac-Conkey's Agar**	Contains bile salts	Used for Entero-bacteriaceae members
3	**Lowenstein Jensen Medium**	Addition of malachite green	Used for M. tuberculosis
4	**Mannitol Salt Agar**	Contains 10% NaCl	Used to recover S.aureus
5	**Crystal Violet Blood Agar**	Contains 0.0002% crystal violet	Used for Streptococcus pyogenes
6	**Thiosulfate citrate bile salts sucrose (TCBS) agar**	Have elevated pH of about 8.5-8.6	Used for isolating Vibrio cholerae
7	**Wilson and Blair's Agar**	Addition of dye brilliant green	Used for recovering S. typhi
8	**Potassium tellurite medium**	Contains 0.04% Potassium tellurite	Used to recover C.diphtheriae
9	**pseudosel Agar (cetrimide agar)**	Contains cetrimide (antiseptic agent)	Used to recover Pseudomonas aeruginosa
10	**Salmonella-Shigella Agar**	Contains bile salts, brilliant green, and sodium citrate	Used for the isolation of Salmonella, which causes typhoid

4. Transport media

- The media transport specimens after collection to **control the overgrowth of organisms.** For the cultivation, this media act as **temporary storage.**
- It also maintains the viability of pathogens in the specimen and prevents them from drying.

Examples of Transport media

✓ Stuart's transport medium (lacks carbon, nitrogen, growth factors). **Cary Blair's transport media** and VR are used to transport feces samples from cholera patients.

✓ Pikes medium helps to transport streptococci from throat patients.

5. Indicator or differential media

- This media shows **visible changes due to the presence of an indicator.**
- It differentiates bacteria based on **colony color growing on the same plate**; biochemical characteristics show organism's growth with chemical indicators like **neutral red, phenol red, methylene blue.**

❑ **Examples of Indicator or differential media**

➢ **Mannitol salt agar (mannitol fermentation shows yellow color colonies);** blood agar is used to differentiate between **hemolytic and non-hemolytic.**

➢ **MacConkey agar** produces **pink colonies** due to lactose utilization and, non-lactose shows pale color colonies.

6. Enrichment media

➢ It is a liquid medium, which also permits the **growth of desired bacteria at a low density.**

➢ The media provides an **environment and conditions** as selective media and **inhibits unwanted bacteria from growing.**

➢ It is for the **isolation of the soil and fecal microorganisms.**

Examples of Enrichment media

✓ **Selenite F-broth** does the isolation of ***Salmonella Typhi*** from a fecal sample. Selenium allows the growth of desired organisms and, detection levels increase for intestinal flora.

7. Storage media

- It maintains the **longevity of bacterial culture**. **Examples are-** cooked meat broth, NA egg saline.

❖ Types of special purpose culture media

1. Assay media

- The media assay vitamins, amino acids, and antibiotics. Example- Antibiotic sensitivity test the media used is **Muller-Hinton agar** has **1.7% agar** for better diffusion of antibiotics.
- It also contains starch, which absorbs toxins released by bacteria. In this media plate **Zone of inhibition** is seen around antibiotics.

2. Minimal media

Principal of minimal media

- Minimal media is a defined **medium with different compositions depending on microorganisms cultured.**
- It contains a carbon source like **sugar/succinate** and inorganic salts like **magnesium, nitrogen, sulfur, phosphorus.**
- **Carbon is a source of energy**; magnesium and ammonium salts are the sources of ions for metabolism stimulation. **Phosphate is a buffering agent.**
- The growth comparison of microbe culture and mutant forms- **Minimal media** and **supplementary-minimal media-** allow the differentiation of wild-type and mutant cells.

Use- The **selection of recombinants**, for the growth of **wild-type microorganisms.**

3. Fermentation media

- The media is for **optimum microorganisms.** Fermentation media **produce high yields** of the product; media **provide energy and nutrients for growth**, and medium gives the substrate for the synthesis of **products in the fermentation.**
- Fermentation media **contains major and minor components-**
- **Major components** – Carbon and nitrogen for energy.

Minor components-

- This **contains inorganic salts**, growth factors, vitamins, buffer, anti-foaming agents, dissolved oxygen, gases, growth inhibitors, enzymes.
- The nutrients in fermentation media depend on the **organism and type of fermentation process.**

Growth media

- It has low nutrients and creates raw material for further fermentation.

Fermentation media

- It has high nutrients and creates end products.
- ✓ **Example-** The yeast requires **1%** carbon, but the fermentation of alcohol, demands **12-13%** carbon in the medium.

❖ ROLE OF FERMENTATION MEDIA

- The media has a **high level of** nutrients, microorganisms, and optimum conditions.
- During the **incubation period under optimum conditions,** microorganisms undergo metabolism.
- Fermentation organisms become **hyperactive** due to **nutrients being in high quantities** and, the result is **nutrients getting consumed, media partially degraded.**
- The waste effluent is the output product. The death of cells occurs if substrate-level reaches the inhibitory concentration and **excess substrate causes them to inhibit vital enzymes.**
- Excess substrate **increases osmotic pressure** and **disturbs enzymatic activity in cells.**
- Microbes release **excess substrate** as partially digested fermentation media and convert it into the **insoluble inert compound** as reserve food, which is **harmless to cells.**
- ✓ **Example- Yeast extract, Beef extract, YPD, BMGY.**

➢ RESUSCITATION CULTURE MEDIA

- The resuscitation method is for the **stressed bacterial recovery**; this is a specialized medium that allows the growth of microbes that have lost the ability to produce because of the **environmental harness.**
- The culture provides nutrients and recovers their metabolism.
  - ✓ **For example-** Bacteria require histamine for growth, and the **medium lacks this component**. Then it inhibits growth.
- The same bacterium is put in a medium having histamine, **then it starts to grow again,** and this medium acts as **resuscitation media.**
  - ✓ **For example-** Tryptic Soy Agar.

➢ APPLICATION OF CULTURE MEDIA

- To culture microbes.
- To identify the cause of infection.
- To identify characteristics of microorganisms.
- To isolate pure culture.
- To store the culture stock.
- To observe biochemical reactions.
- To test microbial contamination in any sample.
- To check antimicrobial agents and preservatives effect.
- To observe microbe colony type, its color, shape, cause.
- To differentiate between different colonies.
- To create antigens for laboratory use.
- To estimate viable count.
- To test antibiotic sensitivity.

UNIT- I
(CHAPTER- 4)
BACTERIA

Points to be covered in this topic

1. INTRODUCTION OF BACTERISA
2. ECOLOGY (HABITAT) OF BACTERIA
3. STRUCTURE OF A BACTERIAL CELL
   - ✓ External Structure of a Bacteria
   - ✓ Internal structure of bacteria
4. SHAPES AND ARRANGEMENT OF BACTERIA
5. CLASSIFICATION OF BACTERIA
   - ✓ Classification of Bacteria based on Gram Staining
   - ✓ Classification of Bacteria based on Oxygen Requirements
   - ✓ Classification of Bacteria based on Optimum Temperature
   - ✓ Classification of Bacteria based on Arrangement of Flagella
   - ✓ Classification of Bacteria based on mode of nutrition

❑ INTRODUCTION

- Bacteria are microscopic, unicellular, prokaryotic organisms. They do not have membrane-bound cell organelles and lack a true **nucleus**, hence are grouped under the domain **"Prokaryota"** together with ***Archae***.
- In a three-domain system, **Bacteria** is the largest domain.
- Bacteria, a **singular bacterium**, is derived from the Ancient Greek word ***"backerion"*** meaning **"cane"**, as the first bacteria observed were bacilli.
- The study of 'Bacteria' is called '**Bacteriology**'; a branch of '***Microbiology***'.

➢ Evolution of Bacteria

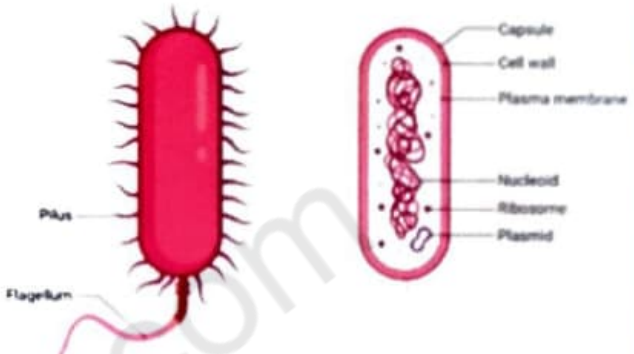
- Bacteria are considered as the **first life-form** to arise on the **Earth about 4 billion years ago**. All other life-forms are evolved from the bacteria.
- A hyper-thermophile of about **2.5 – 3.2** billion years ago was the ancestor of bacteria and archaea that are found in the present time.
- Endosymbiotic association between different bacteria around 1.6 – 2.0 billion years ago give rise to the first proto-eukaryotic cell, which gradually gives rise to eukaryotes.

➢ Ecology (Habitat) of Bacteria

- Bacteria are evolved to adapt and survive in any kind of ecological niches; from normal to **extreme environments**. **Hence, they are ubiquitous.**
- They are found in every possible habitat on the Earth; soil, air, and water.
- They are associated with all the biotic and abiotic components of the Earth. They are essential components of every ecosystem.
- Such bacteria are called **Extremophilic bacteria**. They are found in extreme cold **(Psychrophiles)**, extremely hot **(Thermophiles)**, extreme pH **(Acidophiles and Alkaliphiles)**, extreme pressure **(Barophiles)**, anoxic environments **(anaerobic)**, desertic area **(Xerophiles)**, high radiation area, toxic wastes, barren sand and rocks, deep underground and mountain tip, etc.

- Soil is the most heavily habituated place where they constitute about **0.5% W/W of the soil mass**. One gram of topsoil may contain as many as one billion bacterial cells.
- It is estimated that there are approximately 2×10^{30} bacteria on the Earth, but **only around 2%** of them are fully studied to date.
- Hence, there is a **huge research gap on the diversity and ecology of many unknown bacterial species.**

❑ STRUCTURE OF A BACTERIAL CELL



- **Bacteria are unicellular** i.e. made up of a single cell. They are prokaryotes and their cells are **different from animal and plant cells.**
- In general, the structure of bacteria can be studied as external and internal structures;

❖ External Structure of a Bacteria

It includes a cell wall and all the structures outside the cell wall.

➢ 1. Flagella (sing. Flagellum)

- Flagella are long hair-like filamentous structures of about **4–5 µm** long and **0.01–0.03 µm** in diameter. They confer motility to the bacteria. Flagella are divided into three parts; **filament, hook, and the basal body.**
- The filament is a threadlike part extending outside the cell wall. It is made up of **Flagellin protein.**
- The hook is a short curved structure that joins filament with the basal body. It produces repulsion like the propeller during the revolving of **flagella.**
- The basal body is a **set of rings** embedded in the cell wall and plasma membrane.
- It consists of **2 pairs** of rings in Gram-Negative bacteria and 1 pair of rings in **Gram-Positive bacteria.**
- It **synthesizes polymers of the flagellum,** produces energy for revolution, and regulates movements of the **flagellum.**

Functions of Flagella

- Responsible for motility
- Aids in Chemotaxis
- Aids in bacterial pathogenicity and survival

2. Pili/Fimbriae

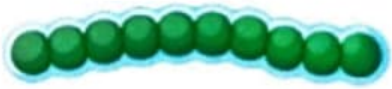
- They are the short, hollow, non-helical filamentous structure of about **0.5 µm in length and 0.01 µm in diameter.** They are exclusively found in **Gram-Negative bacteria.**
- They are composed of **protein 'pilin'** arranged non-helically. They are **short, numerous, and straight than flagella.**
- **Sex pili** are a special kind of pili that take part in bacterial conjugation. They are larger than usual pili; 10-20 µm in length. They are few in number, just 1-4 in number. They are further classified into two types; **F-pili and I-pili.**

Functions of Pili/Fimbriae

- Aids in adherence to host cells
- Sex pili helps in bacterial DNA transfer during bacterial conjugation

3. Capsule

- It is a viscous outermost layer surrounding the cell wall. It is composed of either polysaccharides or polypeptides of both **(~2%)** and water **(~98%).** They are present only in some species of bacteria.
- The capsule is of 2 types; macro-capsule (capsule with a thickness of **0.2 µm** or more) and micro-capsule (capsule with thickness less than **0.2 µm**).



Streptococcus (Capsulated)

- Instead of viscous covering, some bacteria are surrounded by amorphous/ paracrystalline colloidal protein materials called the **slime layer.**

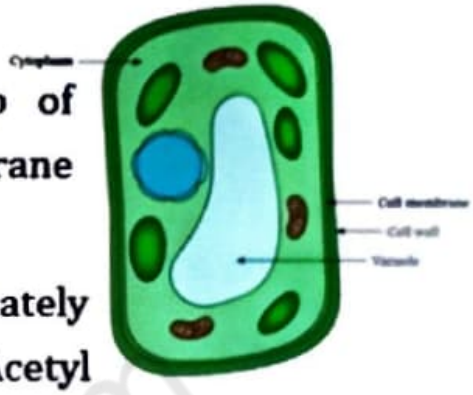
Functions of Capsule

- Aids in adherence
- Prevents from desiccation
- Confer resistance against phagocytosis
- The Slime layer protects from proteolytic enzymes

4. Sheath and Prosthecae

- A sheath is a hollow tube-like structure enclosing chain-forming bacteria, mostly aquatic bacteria. It provides mechanical strength to the chain.
- Prosthecae is a semi-rigid extension of the cell wall and plasma membrane. It increases nutrient absorption and also helps in adhesion.

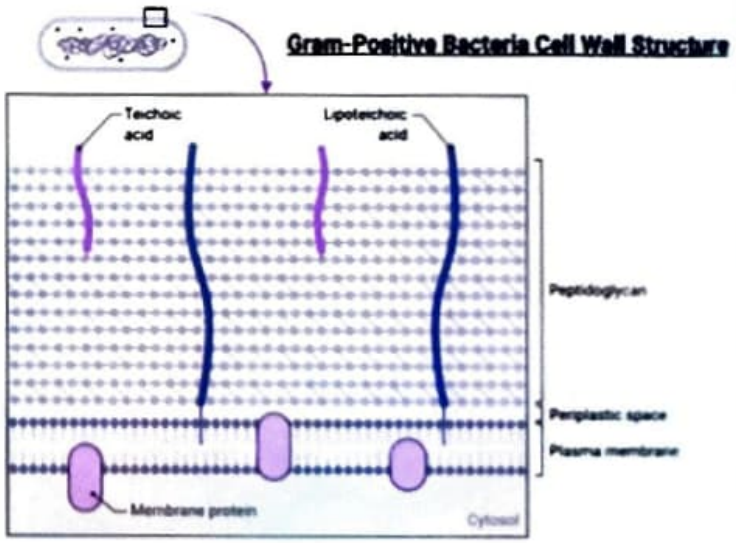
5. Cell Wall



- The cell wall is a rigid structure made up of peptidoglycan that surrounds the plasma membrane as an external coat. It is 10 -25 μm in thickness.
- Peptidoglycan is a cross-linked polymer of alternately repeating N-Acetylmuramic Acid (NAM) and N-Acetyl glucosamine (NAG) polysaccharide sub-units.

Gram-positive cell wall

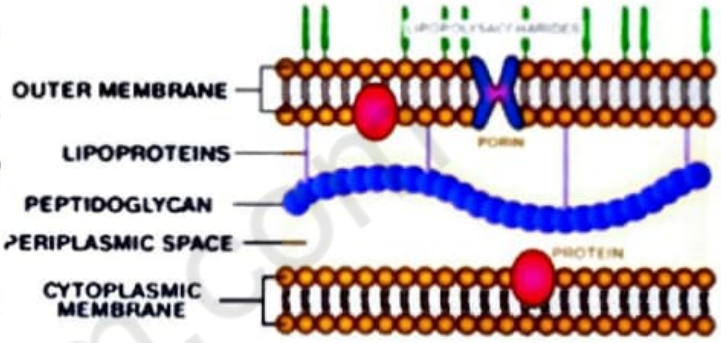
- **The gram-positive cell wall** is a thick cell wall containing a large amount of peptidoglycan, about 40 – 90% of the cell wall, arranged in several layers.
- This type of cell wall also contains acidic sugars like **Teichoic acids**, teichuronic acids, and neutral sugars like mannose, arabinose, Rhamnose, and glucosamine as matrix substances.
- Teichoic acids are made of **polyribitol phosphate or polyglycerol phosphate**. They are major surface antigens of gram-positive bacteria. They are of two types; wall **Teichoic acid and lipoteichoic acid.**



- Teichuronic acid is a polymer of N-acetylmannuronic acid or D-glucuronic acid.
- This type of cell wall takes up the crystal violet dye and confer the purple color of the gram-positive bacteria in Gram staining.

Gram-negative cell wall

- **The gram-negative cell wall** is a thin cell wall with significantly less amount of peptidoglycan.
- It is comparatively more complex than the gram-positive cell wall. It contains lipoprotein, lipopolysaccharide, and outer membrane in addition to peptidoglycan.
- The lipoprotein layer is composed of Braun's lipoprotein. It is embedded in the outer membrane and stabilizes the outer membrane.
- The outer membrane is a **bilayered structure** containing an inner layer resembling the plasma membrane in composition, and an outer layer made up of lipopolysaccharide.



- It is rich in a variety of proteins like **'porin** and outer membrane proteins.
- Lipopolysaccharide is a complex molecule consisting of **3 components; Lipid-A, core oligosaccharide, and O-polysaccharide.**
- Lipid-A is composed of phosphorylated glucosamine disaccharides, long-chain fatty acids, and hydrosymyristic acid.
- Core oligosaccharide is composed of two sugars; **keto-deoxy octanoic acid and a heptose sugar bounded together by Lipid A.**
- **O-polysaccharide** are composed of a wide variety of sugars that differ in between bacterial strains.

Cell-wall of acid-fast bacilli

- It is unique with a large number of mycolic acids. They resist the Decolorization of acid alcohol or sulfuric acid, hence called acid-fast.

Bacteria without a cell wall

- *Mycoplasma* is a minute (50 -300 nm) bacteria without a cell wall. They do not have a fixed shape.
- Besides this natural bacteria, there are several other cell walls deficient forms like protoplasts, spheroplasts, and L-forms.

❖ Gram-Positive Cell-Wall vs Gram-Negative Cell-Wall

GRAM-POSITIVE CELL-WALL	GRAM-NEGATIVE CELL-WALL
Thick (20 – 80 nm)	Thin (10 – 15 nm)
Higher peptidoglycan content	Lower peptidoglycan content
Lower lipid content (2 – 5%)	Higher lipid content (15 – 20%)
The main components are peptidoglycan, teichoic acid, and teichuronic acid	The main components are peptidoglycan, lipoprotein, lipopolysaccharide, outer membrane
Very few amino acids without any aromatic amino acids	Wide variety of amino acids with different aromatic amino acids

❖ Internal structure of bacteria

1. Cell membrane/Plasma membrane

- It is the innermost phospholipid bilayer, just beneath the cell wall, enclosing cytoplasm. It is a **thin (~ 5 -10 nm) semipermeable layer.**
- Unlike eukaryotic plasma membrane, they lack sterols **(except in *Mycoplasma*),** and comparatively have a higher proportion of proteins. In place of sterols, they have sterol-like compounds, **called 'hapanoids'.**
- They contain a wide **variety of fatty acids** like usual saturated and unsaturated types and additionally methyl, hydroxyl, or cyclic groups too.
- The plasma membrane is equipped with several porin proteins for the passive transport of nutrients and ions.

Functions of Cell membrane/Plasma membrane

- ✓ Selective permeability regulates the inflow and outflow of nutrients, ions, and metabolites
- ✓ Electron transport and oxidative phosphorylation

2. Cytoplasm

- It is a colorless, colloidal, viscous fluid with suspended organic and inorganic solutes enclosed within the plasma membrane.
- Unlike eukaryotic cytoplasm, they lack membrane-bound organelles. They have ribosomes, mesosomes, inclusion bodies, nucleic acids floating in them.

2.1 Ribosomes

- Bacterial ribosomes are of 70S type and quite smaller than eukaryotic 80S types.
- They are made up of 2 subunits, the 50S, and 30S. Their main role is to synthesize bacterial proteins and enzymes.
- They are target sites for different antibiotics like erythromycin, macrolides, aminoglycosides, etc.

2.2 Mesosomes

- They are vesicular or branched structures formed by invaginated of the plasma membrane.
- They represent the eukaryotic mitochondria in function and are the site of action of the bacterial respiration enzymes.

2.3 Inclusion bodies

- They are believed to be storage food. They are of two types;

(i) Organic inclusion bodies, containing glycogen or polyhydroxy-butyrate granules, and

(ii) Inorganic inclusion bodies, containing polyphosphate or sulfur granules.

3. Bacterial Nucleus

- They are called nucleoids. Unlike eukaryotic nuclei, they are not enclosed in the nuclear membrane and lack nucleolus and nucleoplasm.
- It is represented by a dsDNA molecule either in a closed circular form or in coiled form.
- Bacterial DNAs are found either in nucleoid as chromosomal DNA or outside nucleoid as a plasmid.

4. Endospore of a bacteria

- Some bacteria under stress form a dormant stage called an endospore. They are produced during unfavorable environmental conditions.
- They have four distinct structural components;

(i) **Core**, containing nucleoid and condensed cytoplasm,

(ii) **Spore** wall, the innermost wall of peptidoglycan,

(iii) **Cortex**, the thickest wall with unusual peptidoglycan.

(iv) **Protein coat**, an outer impermeable layer made of keratin like protein.

❖ Shapes and Arrangement of Bacteria

➢ Basically, bacteria are of four distinct shapes, cocci, bacilli, spiral, and comma-shaped.

A. Cocci shape bacteria

They are spherical bacteria. Based on the arrangement of cells they are further sub-grouped as;

i. **Monococci**; singular cocci. Eg. ***Micrococcus luteus,***

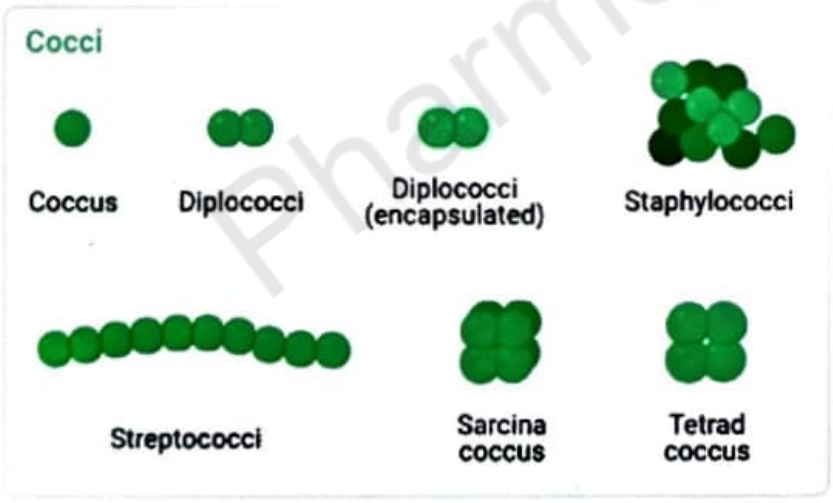
ii. **Diplococci**; two spherical bacteria are arranged in pairs. Eg. ***Neisseria spp., Moraxella catarrhalis, Streptococcus pneumoniae,*** etc.

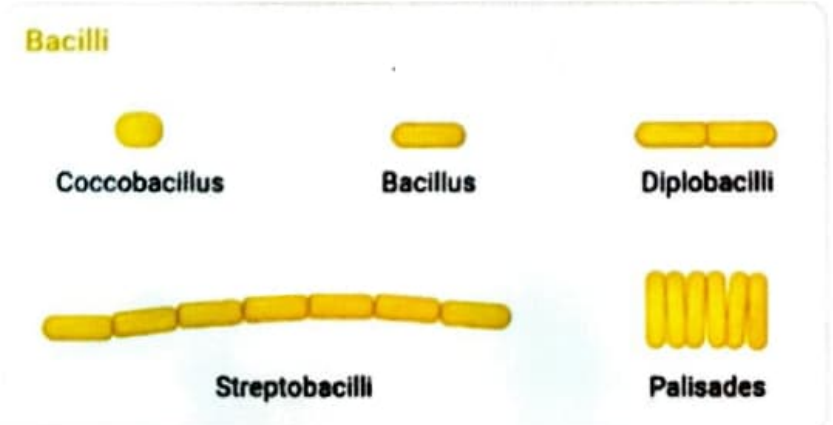
iii. **Streptococci**; spherical bacteria are arranged in a long chain. Eg. ***Streptococcus pyogenes, S. agalactiae,*** etc.

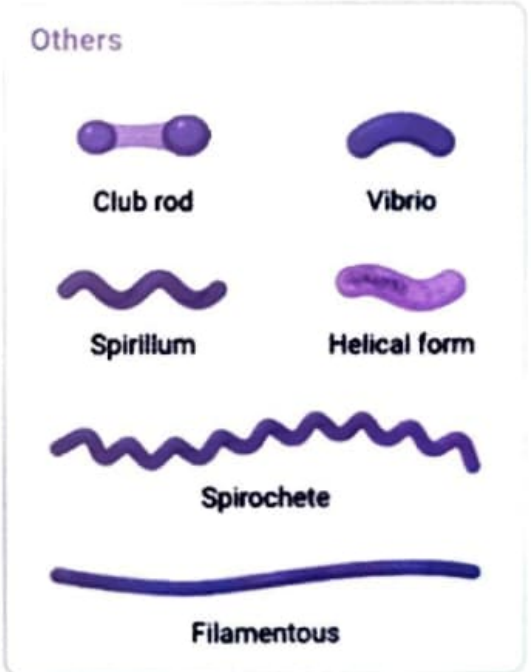
iv. **Staphylococci**; spherical bacteria arranged in irregular clusters like a bunch of grapes. Eg. ***Staphylococcus aureus, S. saprophyticus,*** etc.

v. **Tetrad**; arrangement in a group of 4 cocci. Eg. ***Aerococcus urinae, Pediococcus spp.,*** etc.

vi. **Sarcinae**; arrangement of cocci in a group of 8. Eg. ***Sarcina spp., Clostridium maximum,*** etc.







B. Bacilli shape bacteria

They are rod-shaped bacteria. Based on the arrangement of cells they are also sub-grouped as;

1.Bacillus /Mono-bacillus; single unattached rod-shaped bacteria. Eg. ***Salmonella enterica serovars, Bacillus cereus, etc.***

2.Diplobacilli; bacilli arranged in a pair. **Eg. *Moraxella bovis, Bacillus licheniformis*, etc.**

3.Streptobacilli; bacilli arranged in a chain. **Eg. *Streptobacillus moniliform*, etc.**

4.Palisade; bacilli arranged in fence-like form. **Eg. *Corynebacterium diptheriae*, etc.**

5.Coccobacilli; bacilli with rounded ends or oval-shaped. Eg. *Chlamydia spp., H. influenzae*, etc.

C. Spiral

- They are long helical-shaped or twisted bacteria. Eg. *Spirilla spp. , Spirochetes spp.*, etc.

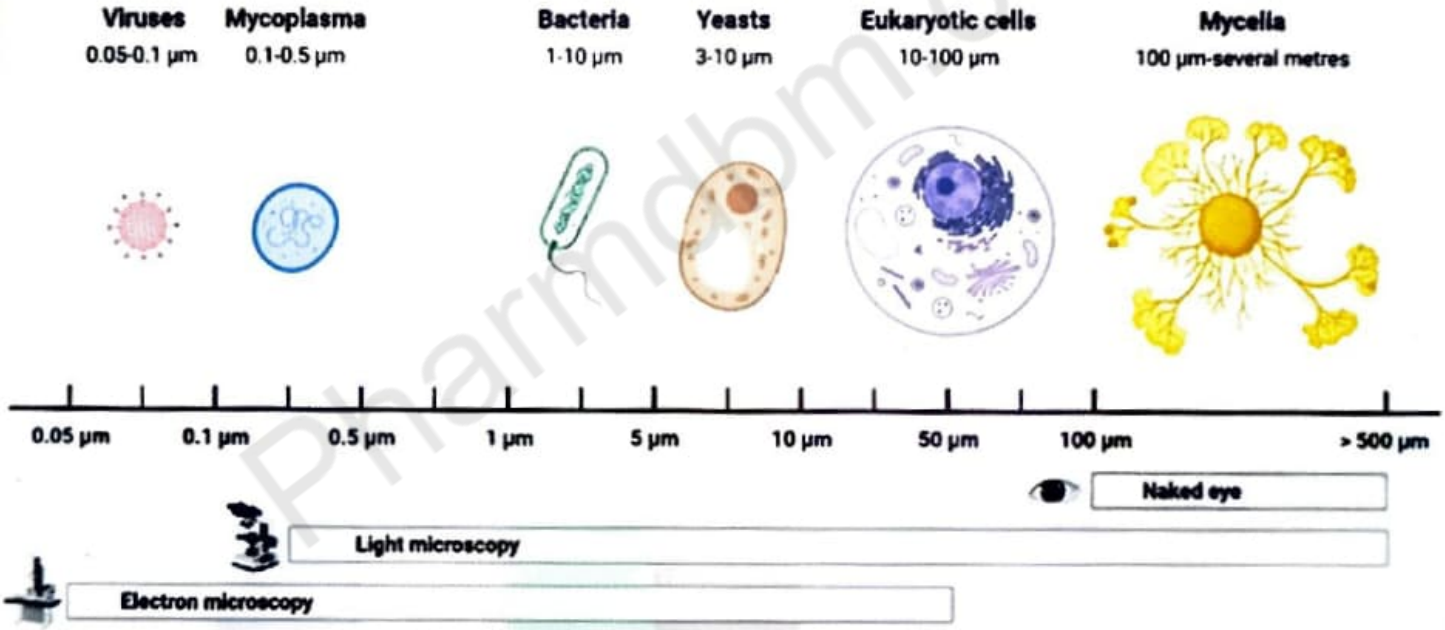
D. Comma shaped

- They are comma (,) like in structure. Eg. *Vibrio spp.* Besides these four basic shapes, several bacteria are found in other shapes like;

1. **Filamentous** (E.g. *Actinobacteria, Candidatus savagella*, etc.)
2. **Star shaped** (E.g. *Stella vacuolata, Stella humosa*, etc)
3. **Appendaged / Budding** (E.g. *Hypomicrobium, Rhodomicrobium*, etc.)
4. **Pleomorphic** (E.g. *Mycoplasma spp.*)
5. **Chinese letter like** (E.g. *Corynebacterium spp.*)
6. **Lobed** (E.g. *Sulfolobus spp.*)
7. **Stalked** (E.g. *Caulobacter crescentus*)
8. **Sheathed** (E.g. *Leptothrix, Clonothrix*)

➢ Size of Bacteria

- Bacteria are microscopic with a wide range of sizes from 0.2 µm to 100 µm.
- Cocci are generally of 0.2 to 1.0 µm.
- Bacilli are generally of 1.0 µm 5 µm in length and 0.5 to 1.0 µm in diameter.
- Spirochetes are generally **20 µm** in length and **0.1 to 1.0 µm** in diameter.
- The smallest bacilli are ***Pelagibacter ubique*** **(370 – 890 nm in length and 120 – 200 nm in diameter).**
- The smallest cocci are ***Mycoplasma genitalium*** with a diameter of **200 – 300 nm.**
- The largest bacteria is ***Thiomargarita namibiensis*** with a diameter of **0.75 mm.**



❑ CLASSIFICATION OF BACTERIA

- There are different schemes for the classification of bacteria. Some of the most common schemes of classifications are:

a. Classification of Bacteria based on Gram Staining

- It is the most common mode of classification used widely in medical and research purposes. Bacteria are grouped into two groups as;

1. Gram-Positive Bacteria

- Bacteria having a **thick peptidoglycan layer and retaining the purple color** of crystal violet during Gram staining are Gram-positive bacteria.
- E.g. ***Staphylococcus, Streptococcus, Enterococcus, Corynebacterium, Streptomyces, Bacillus, Haemophilus, Clostridium, Listeria,*** **etc.**

2. Gram-Negative Bacteria

- Bacteria having a **thin peptidoglycan layer and losing crystal violet but retaining pink / red color** of counterstain safranin during Gram staining are Gram-negative bacteria.
- E.g. ***Escherichia, Salmonella, Shigella, Neisseria, Klebsiella, Proteus, Pseudomonas, Enterobacter, Citrobacter,*** **etc.**

Gram-Positive Bacteria	Gram-Negative Bacteria
✓ Stains violet/purple during Gram staining ✓ Thick cell wall ✓ Thick peptidoglycan layer ✓ Higher mucopeptide and very low phospholipid ✓ Mesosomes present ✓ Fimbriae or pili absent ✓ Forms endospores ✓ Produce exotoxins ✓ Teichoic acid present, ✓ Lack an outer layer	✓ Stains red/pink during Gram staining ✓ Thin cell wall ✓ Thin peptidoglycan layer ✓ Lower mucopeptide and very high phospholipid ✓ Mesosomes absent (rarely present) ✓ Fimbriae or pili present ✓ Forms exospores ✓ Produce endotoxins ✓ Teichoic acid absent, ✓ Presence of an outer layer

b. Classification of Bacteria based on Oxygen Requirements

Bacteria are classified into 3 types as;

1. Aerobic bacteria

- They respire aerobically and can't survive in anoxic environments. E.g. *Pseudomonas aeruginosa, Nocardia spp., Mycobacterium tuberculosis,* etc.

2. Facultative aerobes

- They survive in very low oxygen levels and can survive in both oxygenic and anoxic environments.
- They are Microaerophiles. **E.g.** ***E. coli, Klebsiella pneumoniae, Lactobacillus spp., Staphylococcus spp.,*** **etc.**

3. Anaerobic bacteria

- They respire anaerobically and can't survive in an oxygen-rich environment.
- **E.g.** ***Clostridium perfinges, Campylobacter, Listeria, Bifidobacterium, Bacteroides,*** **etc.**

c. Classification of Bacteria based on Optimum Temperature

Bacteria are classified broadly into 3 types as;

1. Psychrophiles

- They have optimum growth temperature at 15^0C or below.
- E.g. *Chryseobacterium, Psychrobaceter, Polaromonas, Sphingomonas, Alteromonas, Hyphomonas, Listeria monocytogenes*, etc.

2. Mesophiles

- They have optimum growth temperature at 15 – 45^0C. Pathogenic bacteria fall in this category.
- E.g. *E. coli, Staphylococcus aureus, Salmonella Typhi, Streptococcus pyogenes, Klebsiella spp., Pseudomonas spp.*, etc.

3. Thermophiles

- They have optimum growth temperature at above 45^0C.
- **E.g.** *Bacillus thermophilus, Methanothrix, Archaeglobus, Thermophilus aquaticus, Geogemma barosii (at 122^0C), Pyrolobus fumarii* **(at 113^0C),** *Pyrococcus spp.*, etc.

d. Classification of Bacteria based on Arrangement of Flagella

Bacteria are classified into 5 types as;

1. Atrichous

- They are **bacteria without flagella**. E.g. *Lactobacillus spp., Bacillus anthracis, Staphylococcus spp., Streptococcus spp.*, etc.

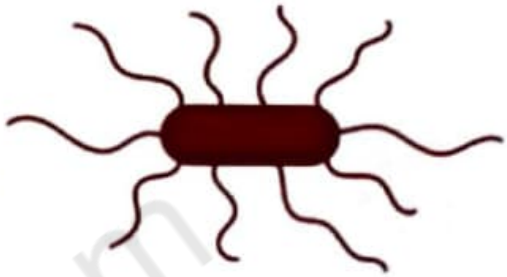
2. Monotrichous

- They are bacteria with only one flagellum at one pole. **E.g. *Campylobacter spp., Vibrio cholerae*, etc.**

3. Lophotrichus

- They are bacteria with multiple flagella at one end. **E.g. *Spirillum, Helicobacter pylori, Pseudomonas fluorescence*, etc.**

4. Peritrichous



- They are bacteria with multiple flagella projecting in all directions. **E.g. *E. coli, Klebsiella, Proteus, Salmonella* Typhi, etc.**

5. Amphitrichous

- They are bacteria with one flagellum at each pole. E.g. ***Alcaligenes faecalis, Nitrosomonas***, etc.

e. Classification of Bacteria based on mode of nutrition

1. Autotrophic bacteria

- They are bacteria capable of assimilating inorganic matters into organic matters i.e. capable of preparing their food like plants. **They are of 2 types;**
- **Photoautotrophs**; They **use energy from sunlight for assimilation.**
- It includes cyanobacteria (*Nostoc, Prochlorococcus*, etc.), purple sulfur bacteria (*Nitrosococcus, Thiococcus, Halochromatium*, etc.), purple non-sulfur bacteria (*Rhodopseudomonas* spp.), green sulfur bacteria (*Chlorobium, Chromatium*, etc.)
- **Chemoautotrophs**; They **use chemical energy for assimilation.** It includes sulfur bacteria (*Beggiatoa, Thiobacillus, Thiothrix, Sulfolobus*, etc.), nitrogen bacteria (Nitrosomonas, Nitrobacter, etc.), hydrogen oxidizing bacteria (*H. pylori, Hydrogenbacter, Hydrogenvibrio marinus*, etc.), methanotrophs (*Methylomonas, Methylococcus*, etc), iron bacteria (*Thiobacillus ferroxidans, Ferrobacillus, Geobacter metallireducens*, etc.)

2. Heterotrophic bacteria

- They are bacteria that derive energy by **consuming organic compounds**, but they **do not convert** organic compounds to inorganics.
- They are parasitic or symbiotic types. **E.g.** *E. coli, Rhizobium spp., Staphylococcus spp., Mycobacterium spp., Klebsiella pneumoniae*, etc.

3. Saprophytic bacteria

- They are bacteria that **decompose organic compounds into inorganic and derive energy.** They are decomposers and feed on dead plants and animals.
- E.g. *Cellulomonas, Clostridium thermosaccharolyticum, Pseudomonas denitrificans, Acetobacter*, etc.

❖ FEEDING IN BACTERIA

- Bacteria feed on **several organic or inorganic compounds**. The food enters the bacterial body either by phagocytosis **(active transport)** or by osmosis and **diffusion or through protein channels (passive transport).**
- They obtain energy by **either photo- or chemosynthesis decomposing organic compounds** or **breaking down inorganic compounds.**
- Based on feeding habits, they are grouped as **autotrophs, heterotrophs, and saprophytes.**

UNIT- I
(CHAPTER- 4.1)
BACTERIA

Points to be covered in this topic

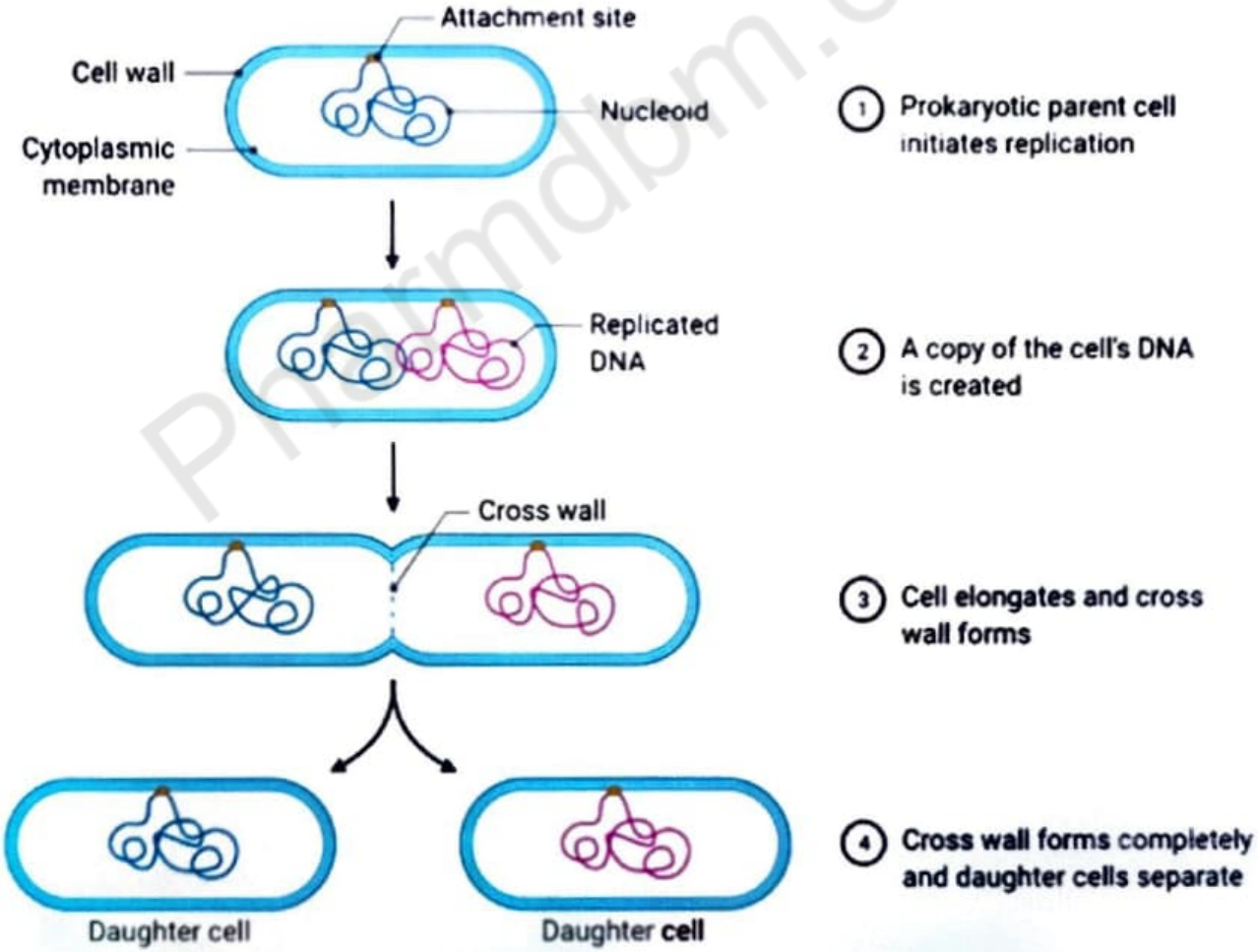
1. REPRODUCTION IN BACTERIA
2. BACTERIAL METABOLISM
3. RESPIRATION IN BACTERIA
4. FERMENTATION IN BACTERIA
5. BACTERIAL DISEASES
6. BACTERIAL IDENTIFICATION
   - ✓ Cultural Methods for Bacterial Identification
   - ✓ Staining and Microscopy for Bacterial Identification
   - ✓ Biochemical Tests for Bacterial Identification
   - ✓ d. Molecular Methods for Bacterial Identification
   - ✓ e. Immunological Methods for Bacterial Identification
7. IMPORTANCE, USES AND APPLICATIONS OF BACTERIA
8. DISADVANTAGES AND LIMITATIONS OF BACTERIA

❑ REPRODUCTION IN BACTERIA

- Bacteria have a **very short generation time** i.e. they reproduce very quickly.
- Their reproduction is **an asexual type** and can be classified into the following types;

1. BINARY FISSION

- It is the most common type. Under **favorable conditions**, each bacterium divides **into two identical bacteria.**
- The bacterial cells first **acquire nutrition grow** at their maximum **size and replicate their DNA.**
- The new replicated DNA called an **incipient nucleus**, migrates towards opposite poles. A transverse septum begins to **develop and separate the two daughter cells.**



2. CONIDIA FORMATION

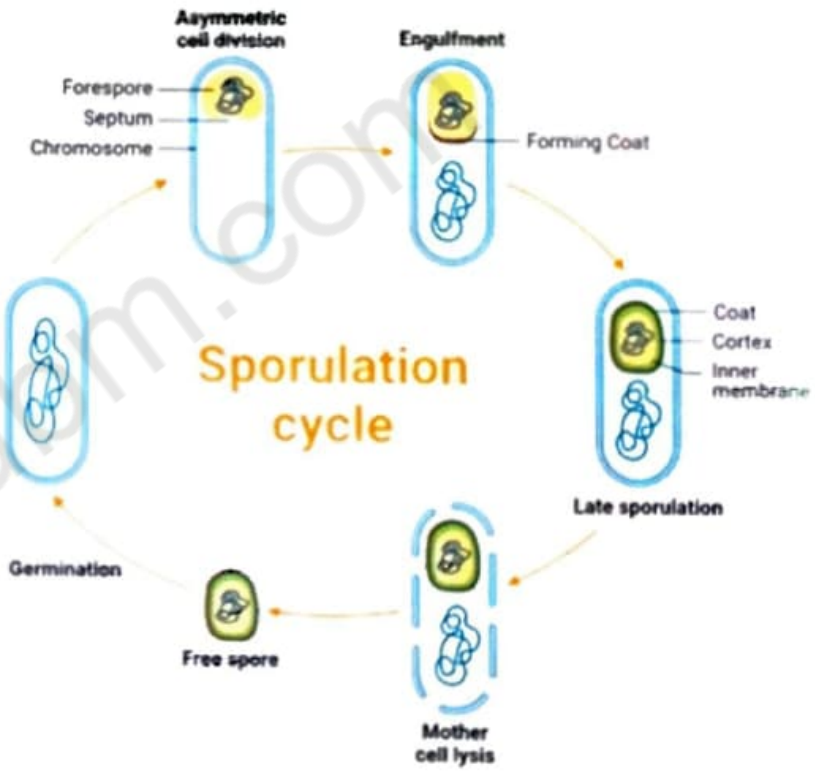
- It is mostly seen in filamentous bacteria like those in actinomycetes e.g. ***Streptomyces, Micromonospora, Rhodomicrobium***, etc.

3. BUDDING

- The bacterial cells develop small swelling, called protuberance or bud, one side. Bacterial DNA replicates and one copy enters into the bud.
- The bud eventually separated and develop into a daughter cell. E.g. ***Planctomyces spp, Rhodomicrobium vannielia, Hyphomicrobium spp.***, etc.

4. ENDOSPORE FORMATION

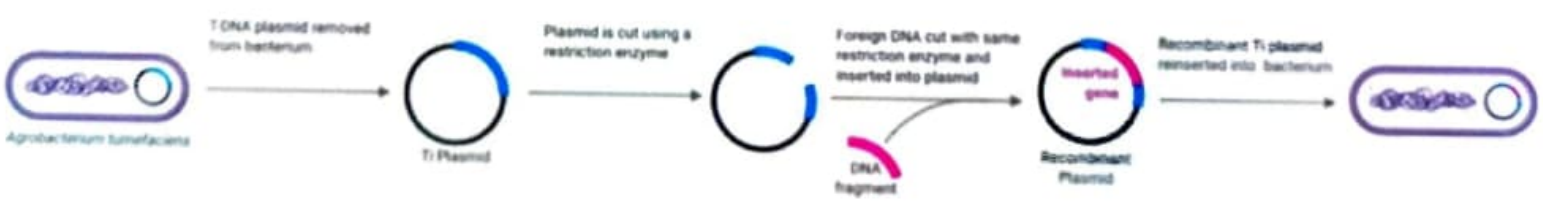
- It is seen in some **Gram-positive bacteria** during **unfavorable conditions and environmental stresses**.
- The cytoplasm becomes concentrated around bacterial **DNA and a thick, hard, and resistant wall develops around it.**



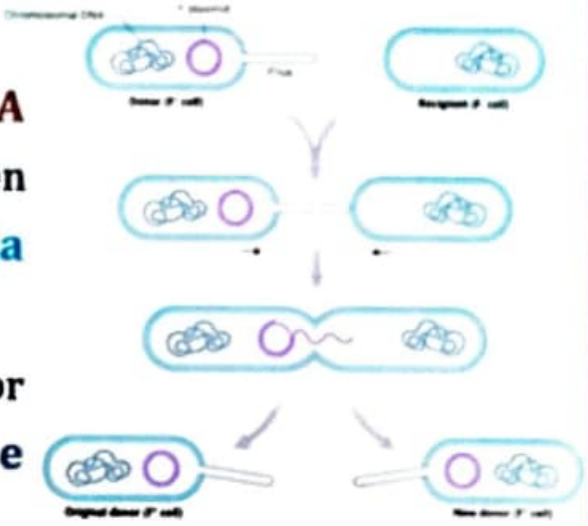
- E.g. ***Bacillus spp., Clostridium spp., Sporosarcina spp.***, etc.

5. TRANSFORMATION

- It is considered a **sexual method**. In this method, the DNA of one bacterium directly enters into a cell of another bacterium of the **same species and forms recombinant DNA.**
- The DNA enters through extracellular environments.



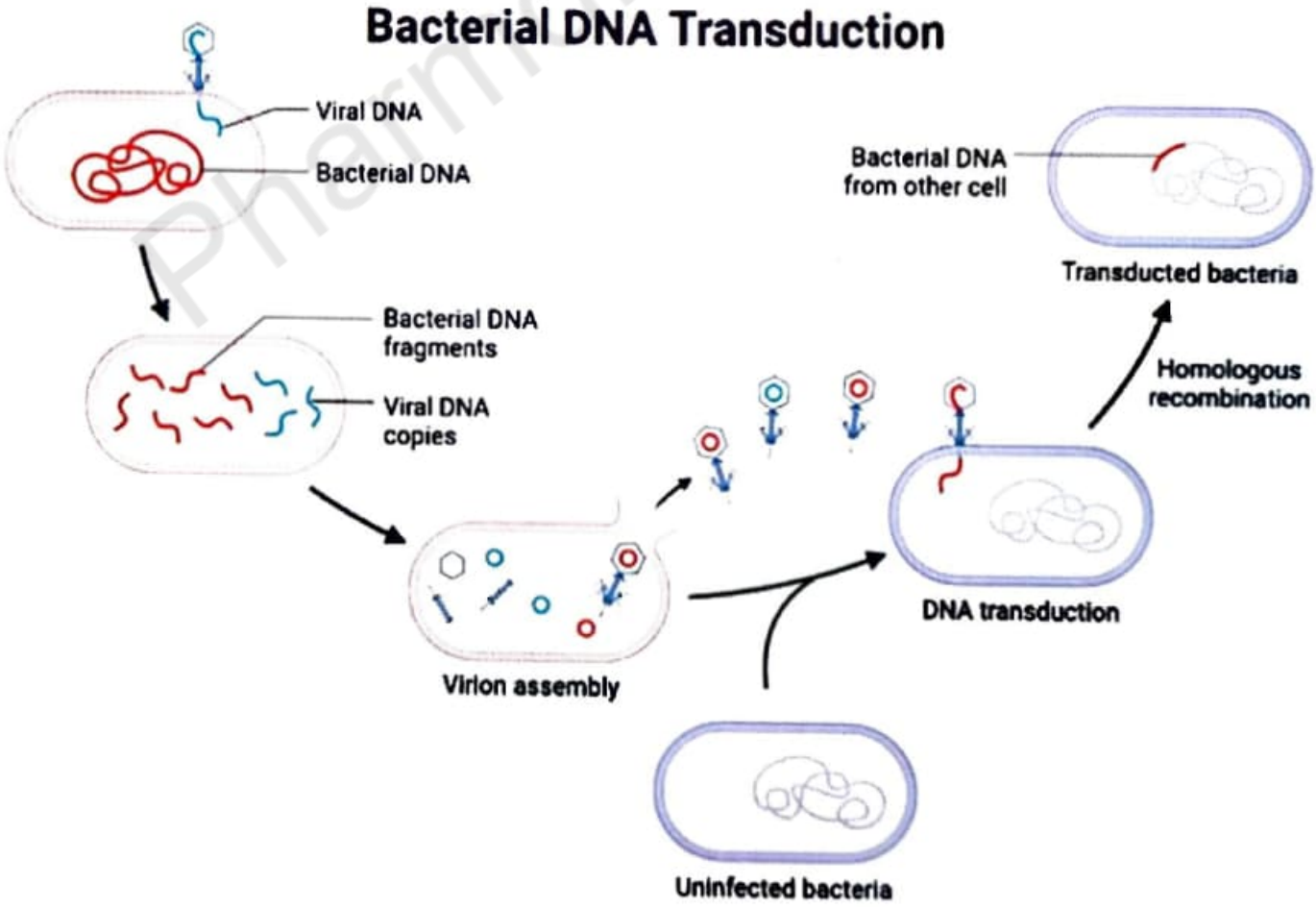
6. CONJUGATION



- It is another **sexual method** where **DNA transformation** is by direct contact between **donor and recipient bacterium via conjugation tube.**
- **Sex pili** are responsible for conjugation. Donor cell develops **sex pilus and attaches to the recipient cell.**
- A conjugation tube or bridge is formed at the **connected point.**
- DNA fragments transform from one bacterium **(donor)** to another **(recipient)** through this tube.

7. TRANSDUCTION

- In this method, **DNA fragments are transformed from donor bacterium to recipient bacterium by bacteriophages.**



➢ BACTERIAL METABOLISM

- It includes all the **metabolic/biochemical activities occurring inside bacterial cells.**
- Based on the **mode of obtaining carbon**, bacterial metabolism can be classified as **heterotrophic metabolism and autotrophic metabolism.**

1. Heterotrophic Metabolism

- In this type, bacteria use **organic compounds** as carbon and energy source.
- Carbohydrates, lipids, and proteins are commonly **oxidized to form ATP and precursor molecules.**
- There are different processes by which bacteria perform heterotrophic metabolism.

2. Autotrophic Metabolism

- In this system bacteria directly **oxidize inorganic compounds (without using solar energy)** to generate energy. It is also called **chemolithotrophy or chemoautotrophy or chemotrophic.**
- The most common metabolic pathways include **Calvin pathway,** reductive TCA cycle, and the acetyl-CoA pathway.
- Based on **different types of inorganic compounds** used as the substrate, there occur different oxidative reactions.
- Common reactions are; **hydrogen oxidation, sulfur oxidation, ferrous oxidation, nitrification, anammox, manganese oxidation, etc.**

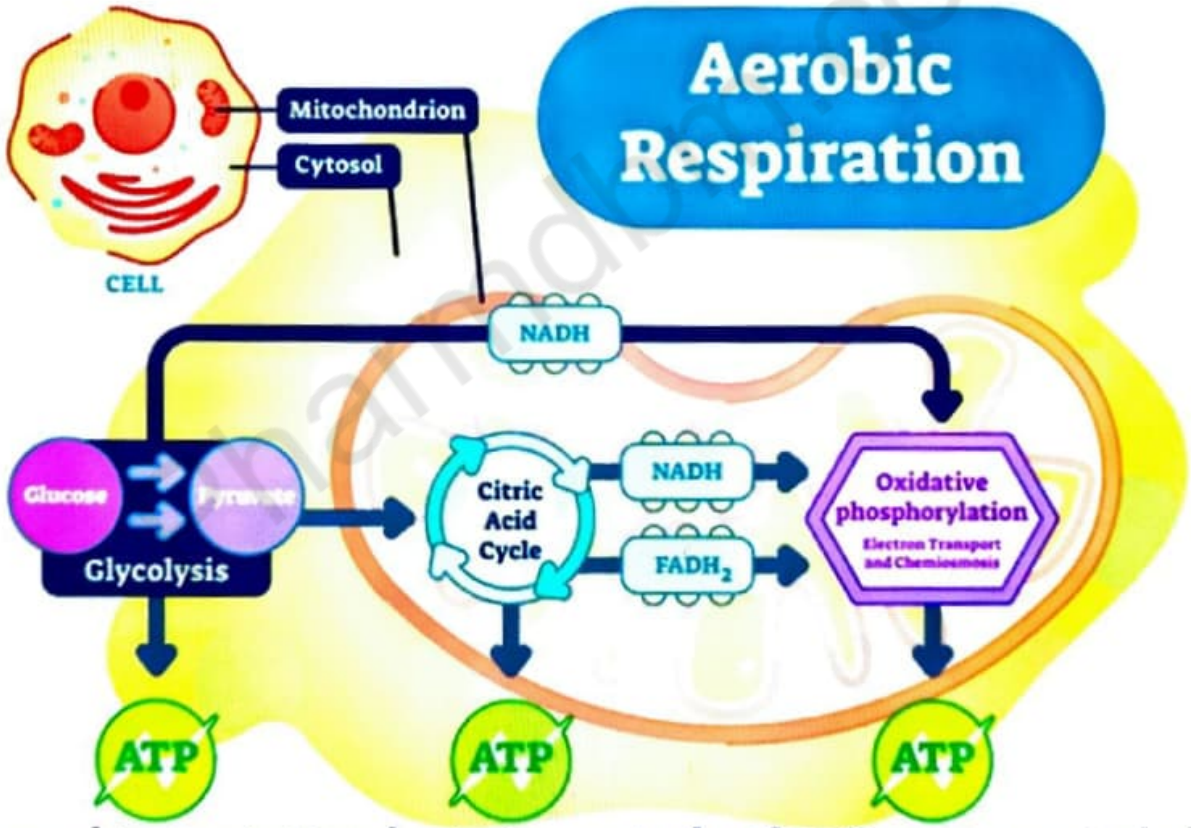
3. Phototrophic Metabolism

- In this system, bacteria use light energy to **oxidize inorganic compounds and produce energy (ATP).**
- There are two types of phototropism in bacteria; **oxygenic and anoxygenic phototropism.**
- In Oxygenic phototropism, **H_2O is oxidized to O_2 to obtain electrons by using light energy.** It is seen in **Cyanobacteria (BGA) containing chloroplast pigment.** Two photosystems(PS), **PS-I and PS-II** are involved in the process.

- In **Anoxygenic phototropism**, H_2S or S_2 or H_2 or other organic compounds are used as **electron donors.**
- It is seen in **Green Sulfur Bacteria**, **Green non-sulfur bacteria**, **purple sulfur bacteria, and purple non-sulfur bacteria**.
- **Only one photosystem is involved**; PS-I in green bacteria and PS-II in purple bacteria.

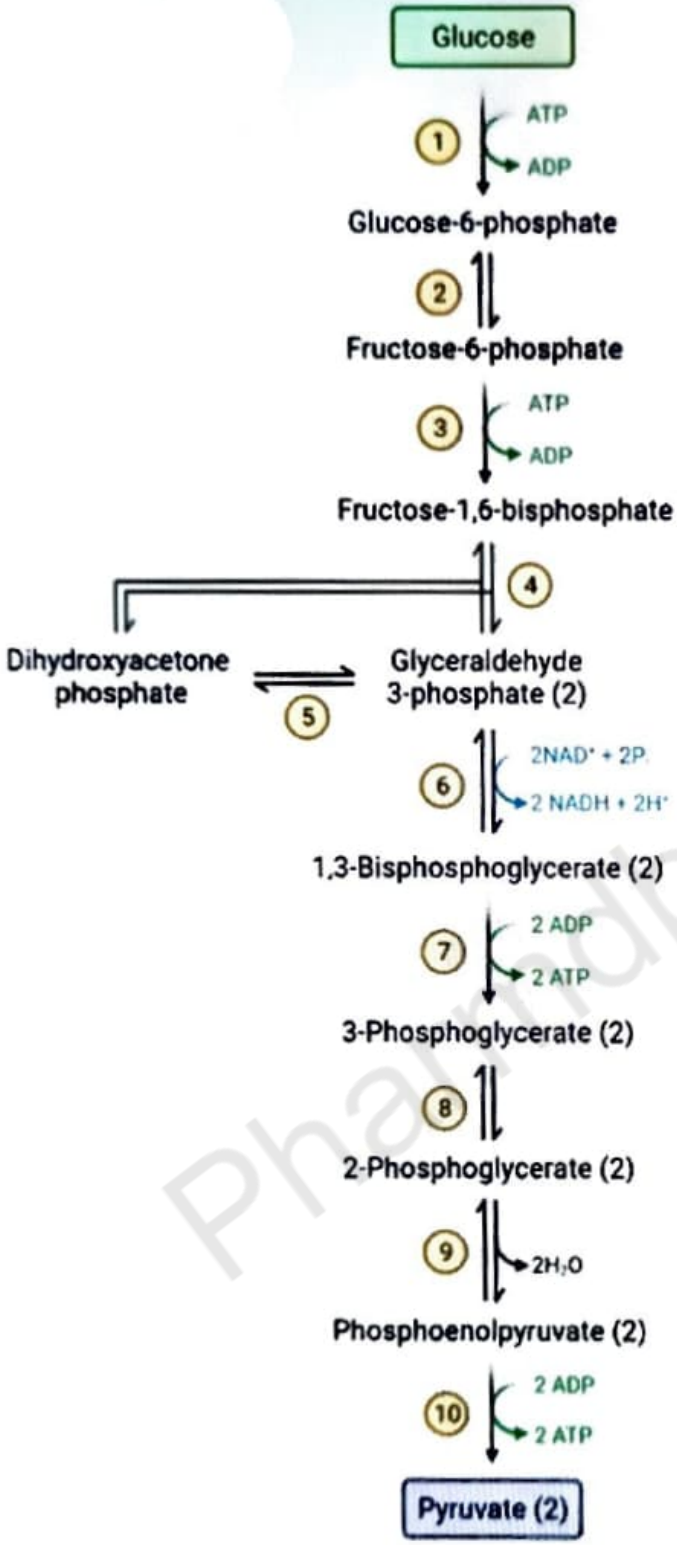
❑ RESPIRATION IN BACTERIA

- It is the process of obtaining energy **(ATP)** by complete oxidation of the food **(glucose)** inside the bacterial cells. Here, glucose completely breaks down into carbon dioxide and water releasing a large amount of energy. **Respiration can be aerobic or anaerobic respiration.**



- In aerobic respiration, bacteria use **molecular O_2** as a terminal electron acceptor. The general reaction occurring in aerobic respiration can be stated as; $C_6H_{12}O_6 + 6O_2 = CO_2 + 6H_2O$ + **energy (38 ATP)**
- In anaerobic respiration, bacteria use nitrate **(NO_3^-)**, sulfate **(SO_4^{-2})**, **CO_2**, fumarate, etc. as terminal electron acceptors. Along with **CO_2 and water, H_2S, and NH_3 are also produced.**

GLYCOLYSIS AND GLYCOLYTIC ENZYMES



ENZYMES	
1	Hexokinase
2	Phosphoglucose isomerase
3	Phosphofructokinase-1
4	Aldolase
5	Triosephosphate isomerase
6	Glyceraldehyde 3-phosphate dehydrogenase
7	Phosphoglycerate kinase
8	Phosphoglyceromutase
9	Enolase
10	Pyruvate kinase

PRODUCTS	
2 ATP	2 Pyruvate
2 NADH	

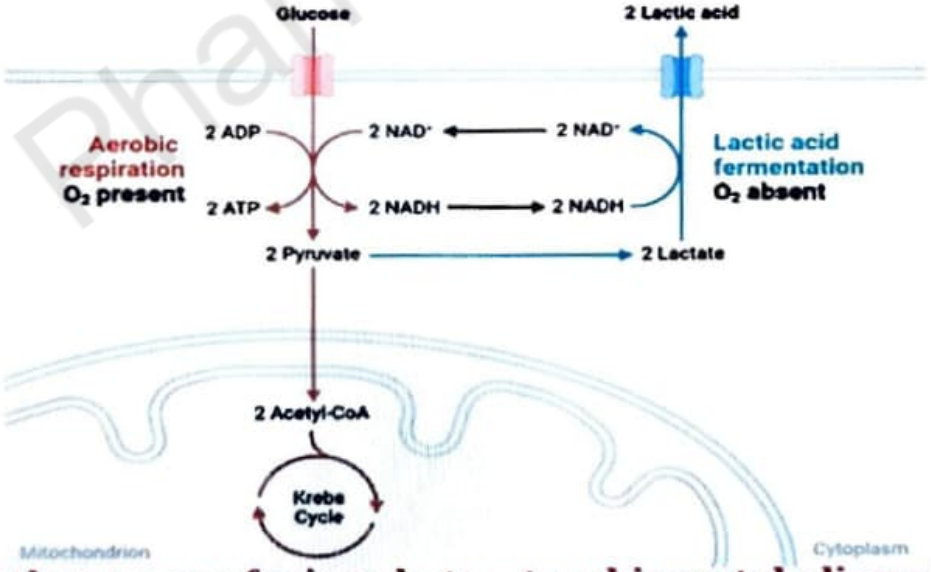
- The complete respiration process includes three basic biochemical pathways; the first is **Glycolytic (Embden-Meyerhof-Parnas / EMP pathway)**, the second is **Tricarboxylic acid (TCA) cycle (Kreb's cycle)**, and the final one is **oxidative phosphorylation (electron transport chain / ETC)**.

- Besides these, there are other minor pathways like the **phosphoketolase pathway, oxidative pentose phosphate pathway (HMP shunt)**, and the **Entner-Doudoroff pathway (ED pathway).**

FERMENTATION IN BACTERIA

- It is the process where **glucose is enzymatically broken down into simpler organic end products like alcohols or acids.**
- Dehydrogenation of glucose **releases energy (ATP)** along with end products. In the homo-fermentation process, **bacteria ferment glucose into a single end product.**
- Mostly glycolytic pathway is followed to produce pyruvic acid, which can be further reduced to acetaldehydes, **-acetolactate, acetyl-SCoA, and lactyl – SCoA.** Some can produce **lactic acid, butyric acid, ethanol, etc.**
- In the hetero-fermentation process, bacteria ferment glucose into a mixture of multiple end products like **ethanol, acetic acid, formic acid, lactic acid, H_2, and CO_2.**
- This type of fermentation is **more common in natural bacterial flora.**

Lactic acid fermentation



There are other types of minor heterotrophic metabolisms like:

(a) Methylotrophy, where bacteria use C1-carbon compounds like **methanol, formaldehyde, methylamines, etc. as an energy source.**

- This is seen in methanotrophs *like **Methylobacter spp., Methylomonas spp., Methylococcus spp.,*** **etc.** Methane is produced during methanogenesis.

(b) **Syntrophy**, where one species of bacteria use the **metabolic end product of another species of bacteria.**

- Here different bacteria pair to achieve a chemical reaction, **which they can't perform individually.**

❖ FACTORS AFFECTING BACTERIAL GROWTH

1. **Water Availability / Water Activity**; bacteria have a **normal** water activity requirement of **0.91 and above.**
   - Water is required for maintaining osmotic pressure, conducting metabolisms, regulating physiology, regulating pH, etc.
2. **Nutrition Level**; different bacteria have different nutritional requirements.
   - Bacteria that require very high nutritional requirements are called **fastidious bacteria.**
   - The bacteria that survive at very low nutrient levels are **called non-fastidious bacteria.**
   - Along with the increase in **nutrition concentration**, bacterial growth increases up to a certain limit, but further increment can't increase the growth rate.
3. **Light intensity**; **phototrophic bacteria require light** for preparing food.
4. **Gaseous concentration**; mostly O_2 and CO_2 influence bacterial growth. Strict Aerobes require high O_2 content.
   - **Facultative aerobes** can grow at very low O_2 content. **Anaerobes** can't survive in an environment with O_2.
5. **Temperature**; different bacteria have a different optimum temperatures for growth.
   - Based on temperature requirements, bacteria are classified as **Mesophiles, Thermophiles, and Psychrophiles.**
   - The most common bacteria, including pathogens, are **Mesophiles** with an optimum temperature of about 37^0C.

6. **Salinity;** salt concentration also affect bacterial growth by **influencing homeostasis and enzymatic actions.**

- **Halophiles** are organisms that have very high optimum ion concentration needed for growth.

7. **pH / Hydrogen Ion Concentration;**

- Bacteria mostly grow in pH **around neutrality (6.5 -7.5).** **Acidophiles** have an optimum pH requirement of **pH below 5.**
- Alkaliphiles have an optimum pH requirement of **pH above 9.** pH affect the enzyme system, proteins, and membrane integrity of bacteria.

❖ BACTERIAL DISEASES

- The bacteria that can cause infection (disease) are called **pathogenic bacteria,** and such **diseases are called bacterial diseases.**
- Most of the bacteria known to us are **non-pathogenic.**
- Only **<5%** are pathogenic. To be pathogenic bacteria, the bacteria must fulfill **Koch's Postulates.**
- Some common bacterial diseases with their **causative species** are listed in the table below.

BACTERIAL DISEASES IN HUMANS	CAUSATIVE AGENT
PULMONARY TUBERCULOSIS	*Mycobacterium tuberculosis*
DIPTHERIA	*Corynebacterium diphtheriae*
CHOLERA	*Vibrio cholerae*
LEPROSY	*Mycobacterium leprae*
PERTUSSIS	*Bordetella pertussis*
TETANUS	*Clostridium tetani*
PLAGUE	*Yersinia pestis*
GONORRHOEA	*Neisseria gonorrhoeae*
SYPHILIS	*Treponema pallidum*
SALMONELLOSIS	*Salmonella enteritis*

❑ BACTERIAL IDENTIFICATION

- This is the method of identifying genera and species of isolated bacteria i.e. to identify **which bacteria are isolated.**
- There are several methods designed and used for bacterial identification.

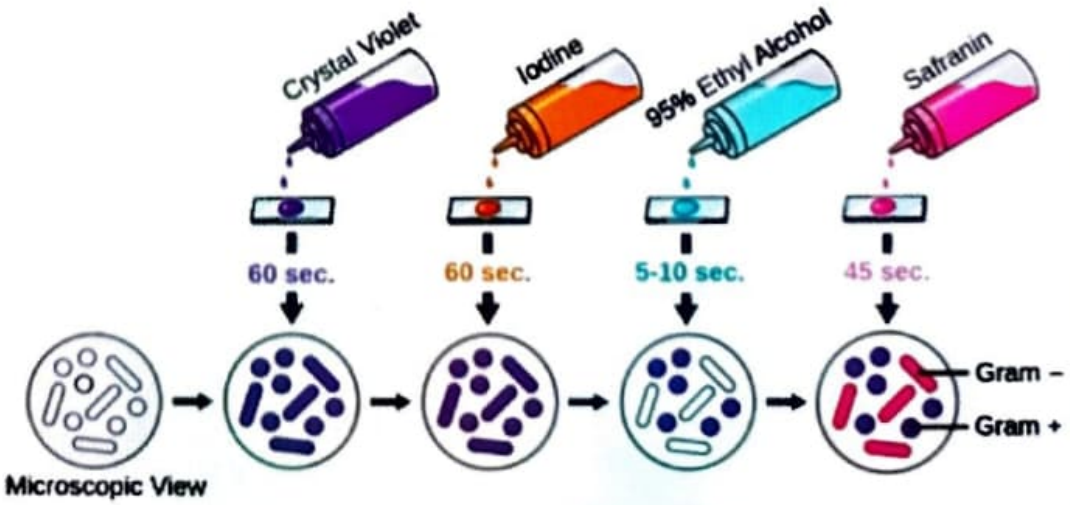
a. Cultural Methods for Bacterial Identification

- It is the method of identifying bacteria by studying their cultural characters in a specific **culture media**. Several **selective and indicator media** are used for bacterial identification. In this method, we study colonial characters like;

I. The shape of colonies (circular, irregular, rhizoid, etc.)
II. Size of colonies (micro, small, medium, large, etc.)
III. Pigmentation
IV. Elevation of colonies (concave, convex, flat)
V. The margin of colonies (smooth, rough, dented, wavy, etc.)

b. Staining and Microscopy for Bacterial Identification

- It is another useful and commonly used method for bacterial identification.
- **Gram staining** is the most important type of staining method used in microbiology for bacterial identification.
- It is a differential staining technique used to differentiate bacteria into two groups; **Gram Positive and Gram Negative**, and to study bacterial morphology.
- Crystal Violet is used as the primary stain, Gram's Iodine is used to fix the CV stain, Acetone/Ethanol is used as a decolorizer and Safranin is used as a counter stain.

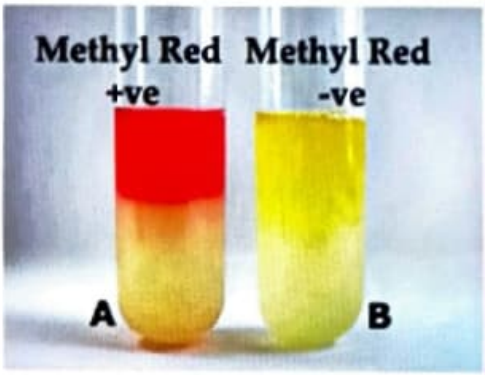


- Besides there are other staining techniques like simple staining, negative staining, Acid Fast Staining (ZN staining), Giemsa staining, Flagella staining, Endospore staining, etc.
- Light microscopy, Fluorescent microscopy, Dark Field microscopy, and Electron microscopy are used.

c. Biochemical Tests for Bacterial Identification

- These tests are the methods of identifying bacteria based on their **biochemical activities.**
- This is a traditional method and is still widely used for the **phenotypic identification of bacteria.**
- **Visual detection of bacterial growth** and color change of media is key to identifying bacteria.
- The principle of biochemical tests is that **different bacteria have different physiology and metabolism,** hence showing different biochemical reactions.

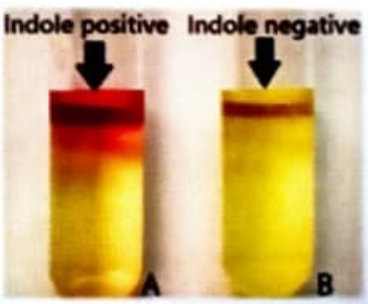
➢ METHYL-RED (MR) TEST



- Used to detect the **production of acid** by **fermentation of glucose** in the medium.
- **MR-VP** broth is used in this test. Bacteria are inoculated in MR-VP broth and incubated overnight. Following incubation, a **methyl red** indicator is added.
- If bacteria ferment glucose in the **medium-producing acid, then the medium will turn red.**

➢ INDOLE TEST

- A qualitative test that detects the ability of bacteria to produce '**indole**' by deamination and hydrolysis of '**tryptophan**' by producing '**tryptophanase**' enzymes.
- It is used to differentiate members of the ***Enterobacteriaceae*** family.
- **Tryptophan-containing media** like Tryptophan broth, Tryptic soy broth, **Sulfide Indole Motility (SIM)** medium, etc. are commonly used in this method.



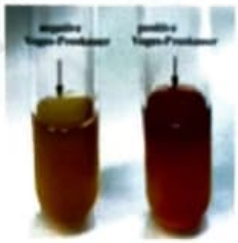
- Bacteria are grown in a medium containing **tryptophan and incubated for 24 hours.**
- Following incubation, an indole reagent is added and the color change is noted. **Development of red or pink color** denoted indole production.

SUGAR FERMENTATION TEST

- Used to study the ability of bacteria to ferment different types of sugars (glucose, lactose, sucrose, mannitol, sorbitol, arabinose, etc.)

VOGES PROSKAUER (VP) TEST

- Used to detect the ability of bacteria to produce neutral products like acetoin or 2, 3-butanediol.
- Bacteria are inoculated in **MR-VP** broth and incubated overnight.
- Following incubation, VP reagents I and II are added and the color change is observed. Development of **cherry red/pink color indicates a positive reaction.**



CATALASE TEST

- Used to detect the ability of **bacteria to produce catalase enzymes.**

CITRATE TEST

- Used to detect the ability of bacteria to utilize citrate as a source of carbon. **Simmon's citrate agar is mostly used.**

OXIDASE TEST

- Used to detect the **ability of bacteria** to **produce the cytochrome oxidase enzyme.**

UREASE TEST

- Used to detect the ability of bacteria to ferment urea to ammonia by producing **urease enzyme.** Urea containing medium like Christensen Urea Agar is used.

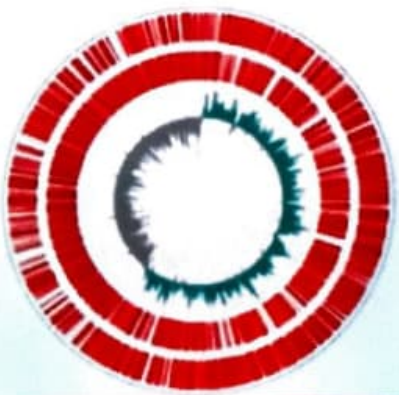
➢ TRIPLE SUGAR IRON (TSI) TEST:

- **Used to detect the ability of bacteria to ferment glucose and lactose or sucrose and release H_2S gas.**
- **TSI agar is used for this test. Color change in slant and butt of TSI agar slant is studied.**
- **Change in color from red to yellow denoted sugar fermentation. If the color of the slant is changed to yellow, it denotes the fermentation of glucose alone.**
- **If the color of the butt is also changed to yellow, it denotes fermentation of either sucrose, lactose, or both. If black coloration is developed, it denoted the production of H_2S.**

❑ **There are several other tests used like;** DNAse test, Nitrate reduction test, Esculin hydrolysis test, Microdase test, Gelatin hydrolysis test, PYR test, ONPG test, Decarboxylase test, Coagulase test, Sulfur reduction test, Starch hydrolysis test, Phenylalanine deaminase test, CAMP test, Bile solubility test, etc.

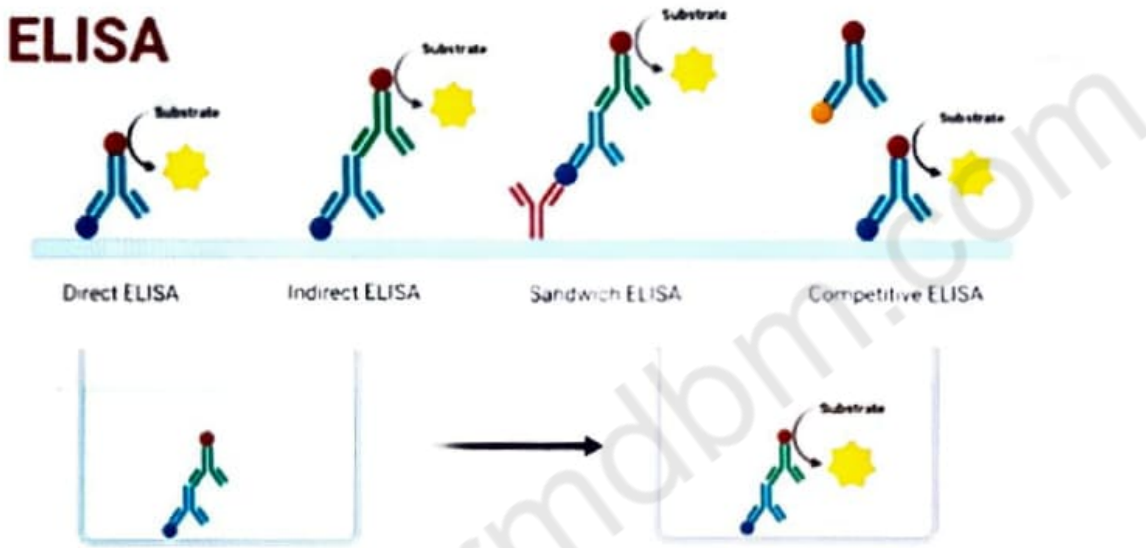
d. Molecular Methods for Bacterial Identification

- **This test includes the study of a bacterial genome and genomic sequences.**
- **This is the most advanced and accurate method used when very precise identification is required.**
- **We can classify bacteria into sub-species, strains, serotypes, or pathovar levels using molecular methods.**
- **This method includes Polymerase Chain Reactions (PCR), DNA / RNA probe tests, Microarray, Electrophoresis, Proteomics, etc.**


e. Immunological Methods for Bacterial Identification

- This method is limited to the **identification of pathogenic bacteria only.** In this method, we **identify bacteria-specific antibodies or antigens in the body of an infected person.**
- The identified **antibody or antigen** is correlated with the identification of the infecting bacteria.
- Enzyme-Linked Immunosorbent Assay **(ELISA),** Radio-immunoassay **(RIA),** Fluoro Immuno Assay **(FIA),** Immuno chromatography tests, etc are commonly used tests.



❑ IMPORTANCE, USES AND APPLICATIONS OF BACTERIA

- They are responsible for **recycling** several nutrients like nitrogen, carbon, sulfur, phosphorus, oxygen, etc.
- They play the most important role in **assimilation and dissimilation of the organic compounds** during any biogeochemical cycle.
- They play a very important role in **regulating atmospheric oxygen levels.** Photosynthetic bacteria (Cyanobacteria, Green Sulfur bacteria) play a very important role in the **production of oxygen during photosynthesis.**
- They are responsible for **biodegradation, composting, decomposition, and bioremediation.**
- They play a very important role **in the management of organic wastes and dead organisms and their parts.**

- Several bacteria are used **industrially for the production of several enzymes.** These enzymes are used in industrial processes, **medical purposes, food processing, etc.**
- Amylase, lipase, cellulases, proteases, hemicellulases, zymase, penicillinases, polymerases, etc. are **produced by bacteria.**
- Bacteria are genetically modified and used in biotechnological applications **to produce hormones like insulin and enzymes.**
- They are used in an anaerobic fermentation process to produce **biogas (methane) which is used as fuel.**
- Different genera of Actinomycetes and other bacteria are the **source of antibiotics used for pharmaceutical purposes.**
- Several bacterial species like *Bifidobacterium, E.coli, Lactobacillus*, etc. are used as **probiotics.**
- Bacteria are used in producing fermented food products like **fermented dairy products, sausages, fermented fruit juices, etc.**
- They are **used in the bioremediation** of oil spillage, xenobiotic, radiation wastes, heavy metal wastes, bio-hazardous wastes, toxic wastes, and other organic and inorganic wastes.
- Bacteria are **used in genetic engineering and molecular research.** Their genes are being used in producing different **Genetically Modified Organisms (GMOs).**
- The bacterial fuel cell is new technology to convert **chemical energy into electric energy.** They can be used as an alternative source of energy.
- In agriculture, they are used as **bio-pesticides, bio-fertilizers, and bio-insecticides.**
- Bacteria are the **pioneer of life forms** in barren lands like deserts, rocks, etc.
- Every living organism living today are evolved from some eukaryotes which were developed from bacteria some **2.0 billion years ago.**

- Bacteria are **present as normal flora in our body**. They help fight against invading pathogens, boost immune response, and help in the **digestion process.**

DISADVANTAGES AND LIMITATIONS OF BACTERIA

- Different pathogenic bacteria are responsible for a **wide variety of human diseases** from simple to life-threatening. Bacterial diseases are responsible for **thousands of death each year.**
- **Bacterial spoilage of foods feeds and pharmaceutical products is another disadvantage**. The **food and pharma industries have to bear huge losses due to bacterial spoilage**.
- Several bacteria like denitrifying bacteria, sulfur-oxidizing bacteria, etc. are **responsible for decreasing the fertility of the soil**, ultimately reducing crop yields.
- **Bacteria can cause disease to crop plants and domestic animals**. This will reduce agricultural production.
- Bacteria cause **deterioration and degradation of useful organic products like furniture, textiles, etc.**

UNIT- I
(CHAPTER- 5)
MICROSCOPES

Points to be covered in this topic

1. PHASE CONTRAST MICROSCOPE

- PRINCIPLE
- OPTICAL COMPONENTS OF PHASE CONTRAST MICROSCOPE (PCM)
- APPLICATIONS
- ADVANTAGES
- DISADVANTAGES

2. DARK FIELD MICROSCOPY

- PRINCIPLE
- COMPONENTS
- USES OF DARK-FIELD MICROSCOPY
- ADVANTAGES OF DARK-FIELD MICROSCOPY
- LIMITATIONS OF DARK-FIELD MICROSCOPY

3. ELECTRON MICROSCOPE

- ADVANTAGES OF ELECTRON MICROSCOPE
- DISADVANTAGES
- PRINCIPLE
- APPLICATIONS

❑ PHASE CONTRAST MICROSCOPE

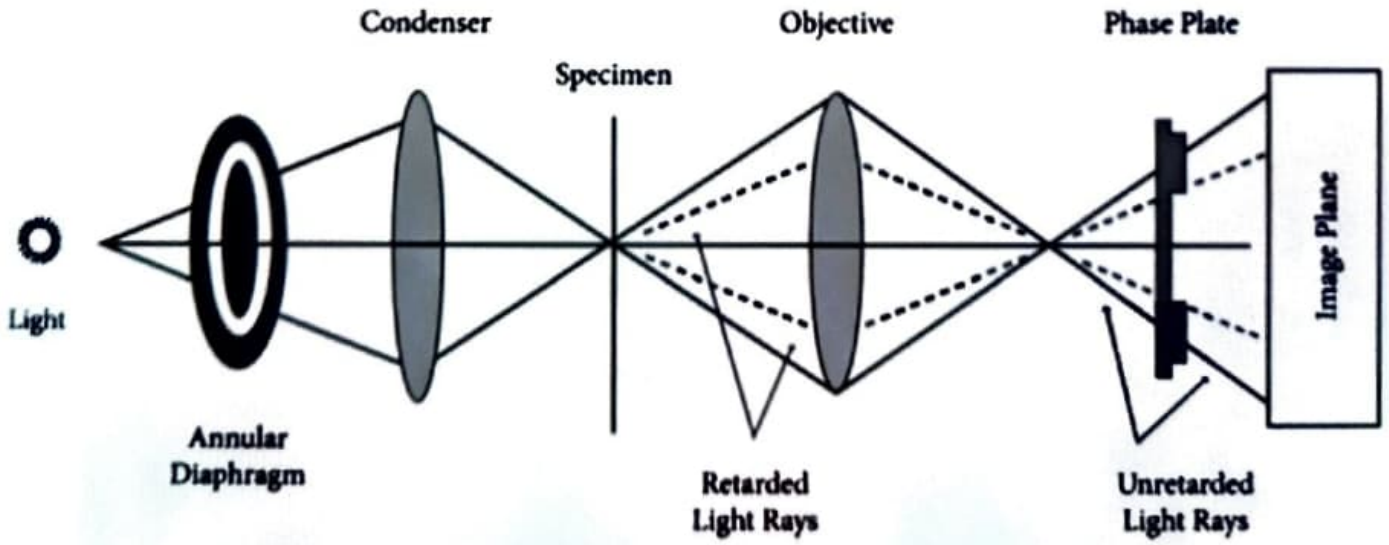
- **Frits Zernike a Dutch Physicist** invented **the Phase Contrast Microscope** and was awarded **Nobel Prize in 1953**. It is the **microscope which allows the observation of living cell.**
- This microscopy uses **special optical components** to exploit fine **differences in the refractive indices of water** and cytoplasmic components of living cells to produce contrast.

❖ PRINCIPLE

- The **phase contrast microscopy** is based on the principle that small **phase changes in the light rays**, induced by **differences in the thickness and refractive index** of the different parts of an object, can be **transformed into differences in brightness or light intensity**.
- **The phase changes are not detectable to human eye** whereas the brightness or light intensity can be easily detected.

❖ OPTICAL COMPONENTS OF PHASE CONTRAST MICROSCOPE (PCM)

- The **phase contrast microscope** is similar to an ordinary compound microscope in its optical components.
- **It possesses** a light source, condenser system, objective lens system and ocular lens system.



Components of phase contrast microscope

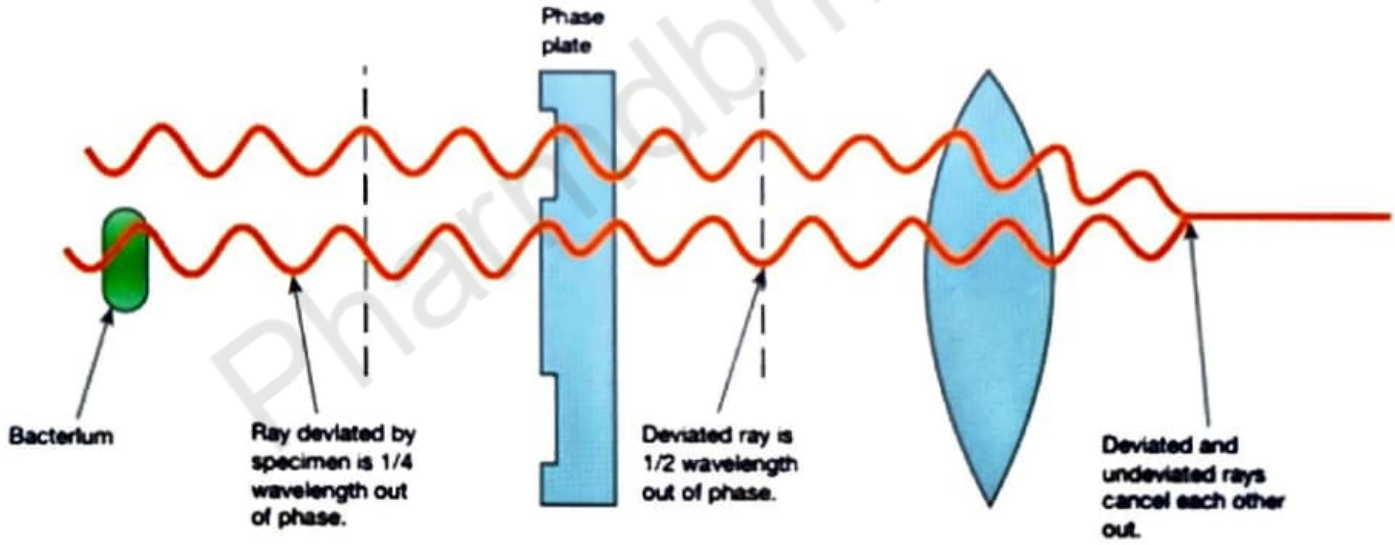
- A phase contrast microscope **differs from bright field microscope in having,**

i. Sub-stage annular diaphragm (phase condenser)

- An **annular aperture in the diaphragm** is placed in the focal plane of the sub-stage which controls the **illumination of the object.**
- This is located **below the condenser of the microscope.**
- This annular diaphragm helps to create a narrow, hollow cone of **light to illuminate the object.**

ii. Phase – plate (diffraction plate or phase retardation plate)

- This plate is located at the back focal plane of the objective lenses.
- The phase plate has two portions, in which one is coated with light retarding material **(Magnesium fluoride)** and the other portion devoid of light retarding material but can absorb light.
- This plate helps to reduce the phase of the incident light.



Production of contrast in phase contrast microscope by phase plate

❖ APPLICATIONS

- It enables the **visualization of living and unstained cells.**
- It is used to **visualization of various cell organelles like mitochondria, nucleus, vacuoles etc.**
- It helps to study cellular events like **cell division, phagocytosis etc.**
- It helps to visualize **cellular movements** like chromosomal **and flagellar movements.**

- It is used to observe growth of the living cells in the plant tissue culture techniques.
- It is used to study membrane permeability of the cells.

❖ ADVANTAGES:

- It provides the clear images of unstained cells.
- It provides high contrast images of the cells.
- It restricts the damages of the cells due to chemical preparation and staining.
- It enhances prolong observation of living cells.
- Its cost is affordable.
- It is widely applied in biological and medical research, especially throughout the fields of cytology and histology.

❖ DISADVANTAGES:

- It produces bright halo surrounding the image because of partial formation of direct and deviated rays.
- It is only effective to observe individual cells.

❑ DARK FIELD MICROSCOPY

- Dark-field describes an illumination technique used to enhance the contrast in unstained organisms or any samples.
- It works by illuminating the sample with light that will not be collected by the objective lens and thus will not form part of the image.
- This produces the appearance of a dark, almost black, background with bright objects on it.

❖ PRINCIPLE

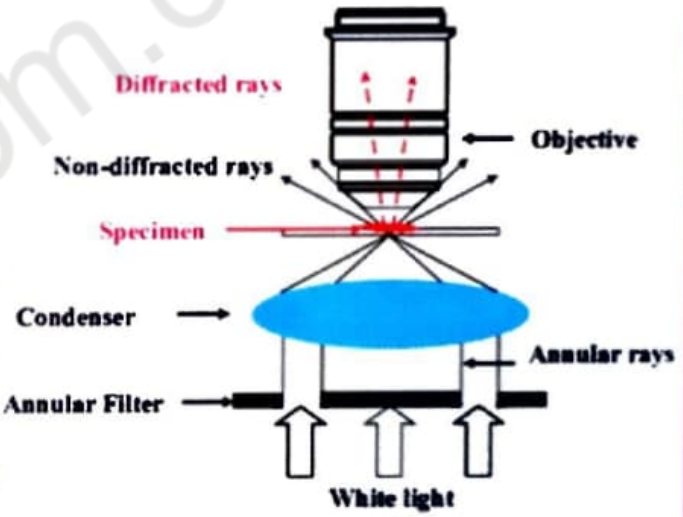
- Dark-field microscopy uses a light microscope with an extra opaque disc underneath the condenser lens, or a special condenser having a central blacked-out area, due to which the light coming from the source cannot directly enter into the objective.

- The path of the light is directed in such a way that it can pass through the outer edge of the condenser at a wide-angle and **strike the sample at an oblique angle**.
- **Only the light scattered by the sample reaches the objective lens for visualization.**
- All other light that **passes through the specimen will miss the objective**, thus the specimen is **brightly illuminated on a dark background.**

❖ COMPONENTS:

- It has dark ground condenser that focuses only the oblique rays of light on to the specimens, high intensity light lamp, a funnel stop that reduces the apparatus of the objective to less than 1.

❖ USES OF DARK-FIELD MICROSCOPY



- Dark-field microscopes are used in the microbiology laboratory for the following purposes;
- Visualization of spirochetes such as ***Treponema pallidum*** (syphilis), ***Borrelia burgdorferi*** (lyme borreliosis) and ***Leptospira interrogans*** (leptospirosis) in clinical samples.
- **Observation of microbial motility**; tufts of bacterial flagella can often be seen in unstained cells by **dark-field or phase-contrast microscopy**
- Observation of internal structure in larger **eukaryotic microorganisms such as algae, yeasts, etc.**

❖ ADVANTAGES OF DARK-FIELD MICROSCOPY

- Resolution by dark-field microscopy is somewhat better than bright-field microscopy

- **Improves image contrast without the use of stain**, and thus do not kill cells.
- Direct detection of **nonculturable bacteria present in patient samples**.
- **No sample preparation is required.**
- **Requires no special set up**, even a **light microscope can be converted to dark field.**

❖ LIMITATIONS OF DARK-FIELD MICROSCOPY

- Necessity to examine **wet, moist specimens containing living organisms very quickly**, because visualization of the **moving bacteria is essential to detection.**
- The sample must be **very strongly illuminated**, which **can cause damage to the sample.**
- Besides the sample, **dust particles also scatter the light and appear bright.**
- Sample material needs to be **spread thinly**, dense preparations can **grossly affect the contrast and accuracy of the dark field's image.**

❑ ELECTRON MICROSCOPE

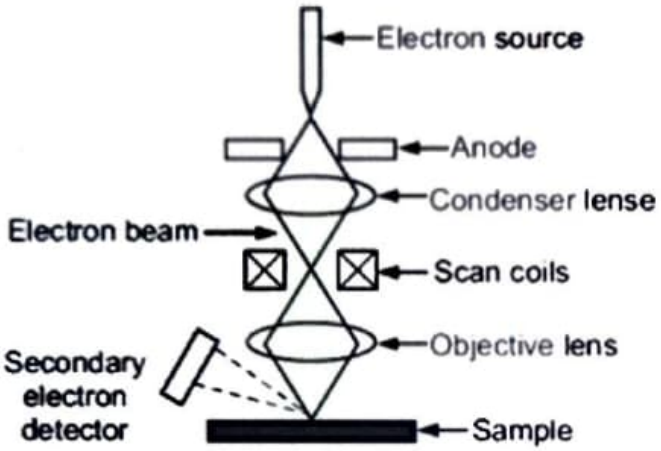
- An electron microscope is a microscope that **uses a beam of accelerated electrons as a source of illumination.**
- The wavelength of an electron can be up **to 100,000 times shorter than that of visible light photons.**
- Electron microscopes **have a higher resolving power** than light microscopes and can reveal the structure of smaller objects.
- **Electron microscope** are used to **investigate the ultra-structure of a wide** range of **biological and inorganic specimen including microorganism, cells, large molecules, biopsy samples, metals and crystals.**
- Modern electron microscopes **produce electron micrograph** using **specialized digital cameras and frame grabbers to capture the image.**

- Electron microscopy is used in conjunction with a variety of ancillary techniques **(e.g. thin sectioning, immunolabeling, Negative staining)** to answer specific questions.
- EM images provide **key information** on the structural **basis of cell function and of cell disease.**
- It is four different types like analytical electron microscopy **(AEM),** scanning transmission electron microscope **(STEM),** Scanning electron microscope **(SEM)** and transmission electron microscope **(TEM).**

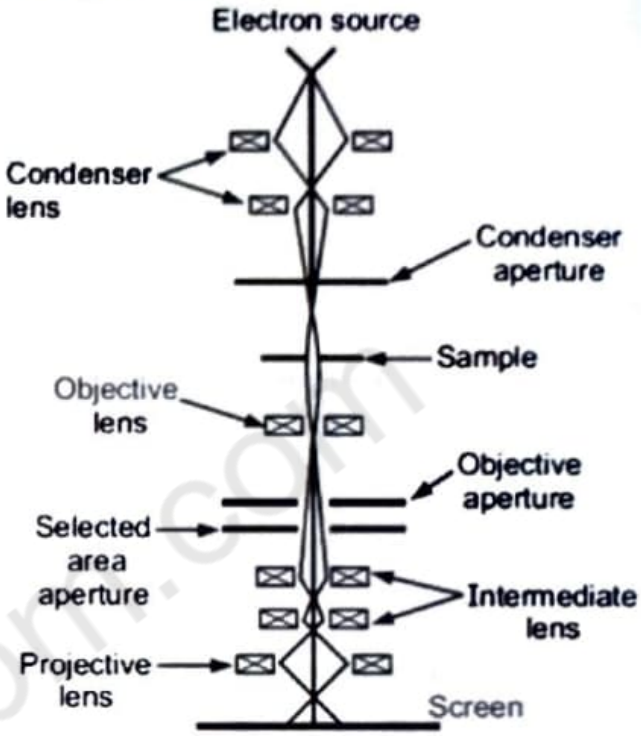
❖ PRINCIPLE

- **AEM** is a type of microscopy for **capturing information on the interaction between the incident electrons and the atoms of the specimen.**
- It is a tool for observing **nano-scale structures** also.
- It is a transmission electron microscope equipped with analytical functions such as **energy dispersive X-ray spectrometry** and electron energy loss spectrometry, **to enable qualitative and quantitative measurements, elemental distribution mapping, and chemical state analyses in the micro-size observation areas.**
- **TEM** is a microscopy technique in which a beam of electrons is **transmitted through an ultra thin specimen,** interacting with the specimen as it passed through it.
- It is mainly used for **cancer research, virology, to examine small column of atom, crystal orientation** etc.
- **STEM** is a modified type of TEM. It uses the **magnetic lenses to focus a beam of electron.**
- The image is formed by the **primary electrons** coming through the specimen.
- **SEM** is a microscopy technique that **produces images of a sample by scanning it with a focused beam of electrons.**

- The electrons interact with the atoms in the sample and produce various signals that contain information about the **sample's surface topography and composition.**
- It is mainly use in **ultra high vacuum**, air and **various liquid states.**
- It is also used for the examination of **live specimen.**



(a) SEM



(b) TEM

❖ APPLICATIONS

Applications of Electron microscope

- Electron microscopes are used **to investigate the ultrastructure of a wide range of biological** and **inorganic specimens including microorganisms, cells, large molecules, biopsy samples, metals, and crystals.**
- **Industrially**, electron microscopes are often used for quality control and failure analysis.
- Modern electron microscopes produce **electron micrographs** using specialized digital cameras and **frame grabbers to capture the images.**
- The science of **microbiology** owes its development to the **electron microscope.** The study of microorganisms like bacteria, virus, and other pathogens have made the treatment of diseases very effective.

❖ ADVANTAGES OF ELECTRON MICROSCOPE

- Very **high magnification**
- **Incredibly high resolution**
- Material rarely distorted by preparation
- It is **possible to investigate a greater depth of field**
- **Diverse applications.**

❖ DISADVANTAGES

- Instruments are **highly expensive.**
- The electron microscope is dynamic, which means that the **voltage needs to be highly stable.**
- The cooling system needs constant circulation pumping through the unit and the **vacuum setup also requires** consistent pressure and continuous pumping to keep the entire system in proper working condition.
- An electron microscope requires that all samples be viewed in a **vacuum**. Otherwise, the molecules that occur naturally in the **air would scatter and distort the electrons.**