B.Sc. MICROBIOLOGY AND CLINICAL LAB TECHNOLOGY

I YEAR

I SEMESTER

GENERAL MICROBIOLOGY

COURSE CODE: 7BMC1C1

Dr. Umayal Ramanathan College for Women

Accredited with B+ Grade by NAAC
(Affiliated to Alagappa University
Sivagangai,Karaikudi – 630003
Tamil Nadu,India

CORE COURSE - I – GENERAL MICROBIOLOGY

Course outcomes

Semester	Course Title	Course Code	Course Outcome	
I	General Microbiology	7BMC1C1	CO1 .	Learn the basics of microbiology and recite the development of Microbiology, concepts contributions of Microbiologists and their inventions, Classification of Microorganisms based on microbial kingdoms
			CO2	Understand the concept of Microscope Principles and applications
			CO3	Understand the ultrastructure of bacteria, fungi, actinomycetes, protozoa and viruses
			CO4	Study in detail about Principles and methods of Sterilization,Chemotherapy antimicrobial resistance, disinfectants and Culture media
			CO5	Understand the growth cycle f bacteria, factors affecting growth and transport mechanisms

CONTENT DELIVERY

Sem-I	Part	Course Code	Title of the Course	Cr.	Hrs./ Week	Max. Marks		
						Int.	Ext.	Total
	III	7BMC1C1	**Core – I** -General Microbiology	4	6	25	75	100

B.Sc. MICROBIOLOGY AND CLINICAL LAB TECHNOLOGY

I YEAR – I SEMESTER

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CORE COURSE - I – GENERAL MICROBIOLOGY

Unit I

Definition and scope of microbiology – History and recent development – Spontaneous generation. Contributions of Louis Pasteur, Leewenhoek, Lazaro Spallanzani, John Tyndall, Joseph Lister, Alexander Fleming and Kary B Mullis. Microbial Kingdoms- Haeckel's Three Kingdom and Whittaker's Five Kingdom concept.

Unit II

Microscope Principles and applications – Simple, compound light microscopy – Phase contrast – Fluorescence – Electron microscopy (TEM and SEM). Staining- Principles and techniques - Simple staining, Gram staining, Capsule staining, Spore staining and Acid fast staining.

Unit III

General characteristics and Ultra structure of bacteria: Subcellular structures- cell envelope, slim layer, capsule, cell wall composition (Gram positive and Gram negative) and cell inclusions. Bacterial reproduction. General characteristics of algae, fungi, Actinomycetes, protozoa and virus.

Unit IV

Principles and methods of Sterilization: Physical methods (Heat, Filtration and radiation) and Chemical methods. Chemotherapy – antibiotics – source – classification – mode of action – antimicrobial resistance, disinfectants. Culture media: Selective / differential media – enrichment media.

Unit V

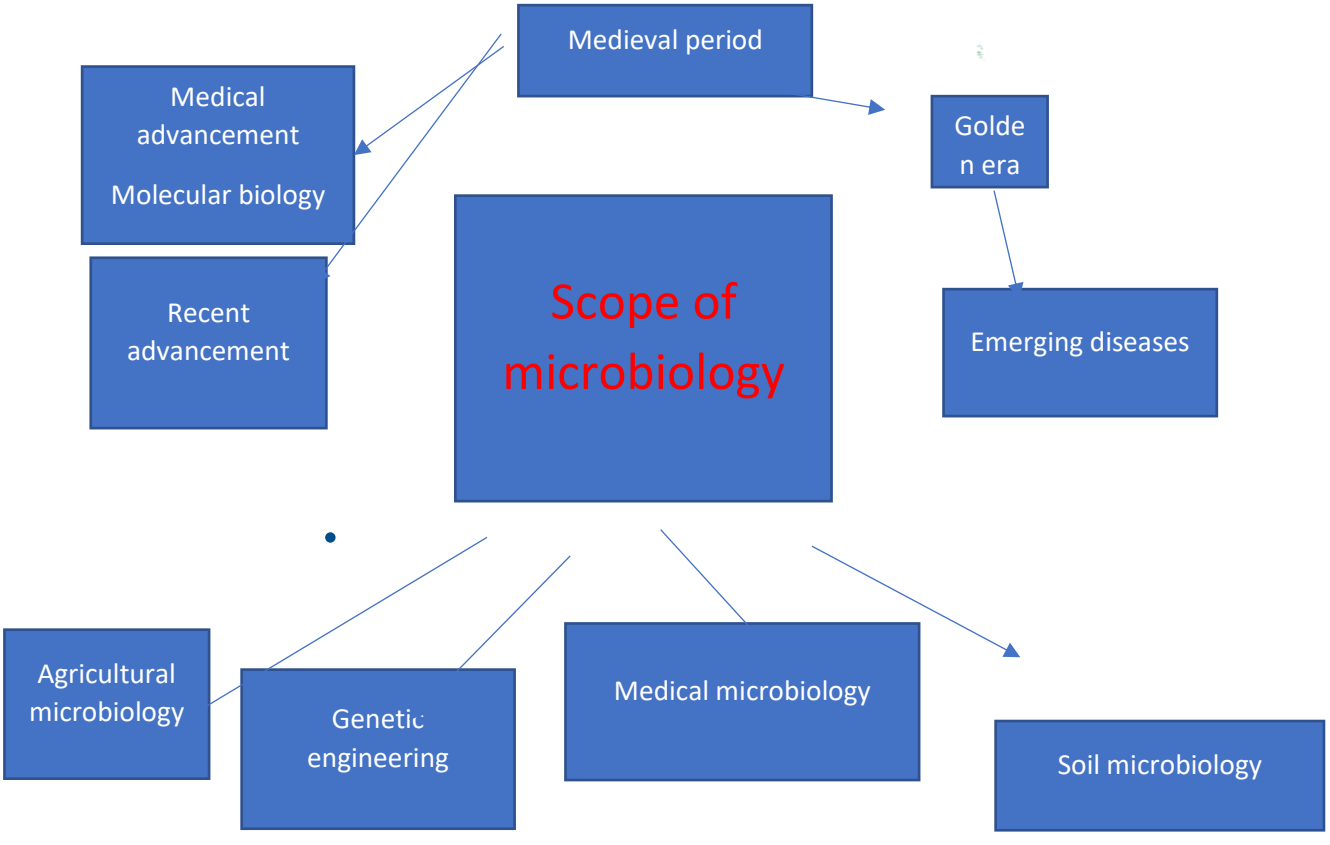
Bacterial Growth curve – Lag Phase, Exponential Phase and decline Phase. Factors influencing and affecting microbial growth – pH, temperature and light. Nutritional groups of bacteria Transport of nutrients by active and passive transport.

Books for Reference:

1. Gerard J. Tortora and Berdell R. Funke (2016) Microbiology, An Introduction, 12th edition
2. Prescott, Harley, Klein, 2011, Microbiology – International Edition, eighth Edition, Published by McGraw-Hill Education , New York,
3. Pelczar M.J, Chang E.C.S, Krieg N.R. Microbiology, Fifth edition, McGraw Hill Company, Newyork.
4. Stanier, RY., et al., General Microbiology, 5th ed. Macmillan Press.

Mindmap


Pasteurization
Fermentation
Vaccines
Koch
Germ theory
Joseph Lister
Typ
Francisco Redi
Lazzaro Spallanzani


Medieval period
Medical advancement
Molecular biology
Golde n era
Recent advancement
Scope of microbiology
Emerging diseases
Agricultural microbiology
Genetic engineering
Medical microbiology
Soil microbiology

UNIT-1

INTRODUCTION AND SCOPE OF MICROBIOLOGY

Introduction

Microbiology is the science that deals with the study of microorganisms. The term microbiology derives its name from three Greek words *mikros* [small] bios [life] and logos [study]. Microorganisms are tiny and invisible to naked eye. So, they can be looked into and studied only with the help of microscope.

Small subcellular or cellular living beings with milli-micron or micron in size and are not visible to our naked eyes are called micro-organisms.

Micro-organisms are basically classified under the following 2 groups:

1. **Prokaryotic microbes:**

   These include subcellular living entities like prions, viroid, viruses and cellular organisms like bacteria, cyanobacteria etc.

2. **Eukaryotic microbes:**

   These include cellular microbe belonging to following groups-

a. Algae. Ex: Chlamydomonas, Diatoms.

b. Fungi. Ex: Yeast, Rhizopus.

c. Protozoans. Ex: Plasmodium, Amoeba.

Micro-organisms are commonly called microbes and they were the first to occupy planet earth even before man and other creatures. Microbes are present in every part of biosphere

HISTORY OF MICROBIOLOGY

Although microbes were the first life forms to occupy the planet earth, the knowledge about microbiology is well developed with new dimension only after the invention of microscopes and

contribution of knowledge to the field of microbiology from various scientists.

Table 1.1 Some Important Events in the Development of Microbiology

Date	Microbiological History	Other Historical Events
1546	Fracastoro suggests that invisible organisms cause disease	Publication of Copernicus's work on the heliocentric solar system (1543)
1590–1608	Jansen develops first useful compound microscope	Shakespeare's *Hamlet* (1600–1601)
1676	Leeuwenhoek discovers "animalcules"	J. S. Bach and Handel born (1685)
1688	Redi publishes work on spontaneous generation of maggots	Isaac Newton publishes the *Principia* (1687)
		Linnaeus's *Systema Naturae* (1735)
		Mozart born (1756)
1765–1776	Spallanzani attacks spontaneous generation	
1786	Müller produces first classification of bacteria	French Revolution (1789)
1798	Jenner introduces cowpox vaccination for smallpox	Beethoven's first symphony (1800)
		The battle of Waterloo and the defeat of Napoleon (1815)
		Faraday demonstrates the principle of an electric motor (1821)
1838–1839	Schwann and Schleiden, the Cell Theory	England issues first postage stamp (1840)
1835–1844	Bassi discovers that silkworm disease is caused by a fungus and proposes that many diseases are microbial in origin	Marx's *Communist Manifesto* (1848)
1847–1850	Semmelweis shows that childbed fever is transmitted by physicians and introduces the use of antiseptics to prevent the disease	Velocity of light first measured by Fizeau (1849)
		Clausius states the first and second laws of thermodynamics (1850)
1849	Snow studies the epidemiology of a cholera epidemic in London	Graham distinguishes between colloids and crystalloids
		Melville's *Moby Dick* (1851)
		Otis installs first safe elevator (1854)
		Bunsen introduces the use of the gas burner (1855)
1857	Pasteur shows that lactic acid fermentation is due to a microorganism	
1858	Virchow states that all cells come from cells	Darwin's *On the Origin of Species* (1859)
1861	Pasteur shows that microorganisms do not arise by spontaneous generation	American Civil War (1861–1865)
		Mendel publishes his genetics experiments (1865)
		Cross-Atlantic cable laid (1865)
1867	Lister publishes his work on antiseptic surgery	Dostoevski's *Crime and Punishment* (1866)
1869	Miescher discovers nucleic acids	Franco-German War (1870–1871)

continued...

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).

This Creature Could Be the 'True Intermediary' Able to Walk Like a Man and Climb Like an Ape

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, he is considered as the "Father of microbiology".
- 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 spontaneous generation which includes experimentations 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 goosenecked flask. When he boiled broth in a flask with a straight neck and left it exposed to air, organisms grew. 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. 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 Tyndallisation.
- 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:

- The organism causing the disease can be found in sick individuals but not in healthy ones.
- The organism can be isolated and grown in pure culture.
- The organism must cause the disease when it is introduced into a healthy animal.
- 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 (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.

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 (a disease transmitted to people by bites of dogs and other animals). 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 (WBC) 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.

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 reorted 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 (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 Sakahiro 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).
- Antibiotics were discovered completely by accident in the 1920s, when a solid culture in a Petri dish (called a plate) 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.

n 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

Microscope invented by Antony Van Leeuwenhoek

Contributions of Antony Van Leeuwenhoek

- He was Dutch Philosopher, born on 24 October 1632.
- He is regarded as Father of „Bacteriology" and „Protozoology", because of his contribution to the field of bacteria and protozoa.
- He invented simple microscope having magnification power up to 300X.
- He observed bacteria from his teeth scrap under the microscope invented by him and he named them as „animalcules".
- He also discovered bacteria in rain water ditch and protozoans like paramecium and amoeba.
- He presented all his observations with illustration before scientist organization „Royal Society of London" in 1683.

Contributions of Louis Pasteur

- He was a French Biochemist, born on 27 December 1822.
- He is regarded as „Father of Microbiology and Immunology".
- He proposed the „Theory of Germ Disease", where diseases of plants, viruses, animals and human beings are caused by pathogenic microbes.
- He disproved the theory of abiogenesis by conducting „Swan neck flask experiment".
- He discovered the presence of bacteria in the air and classified the bacteria into aerobic and anaerobic forms.
- He coined the term „microbiology", aerobic, anaerobic.
- He discovered the role of anaerobic microbes in the fermentation of sugar.
- He developed technique to prevent souring of milk and spoilage of wine. His technique is now called

Pasteurization technique.

- He first isolated bacteria causing cholera (*Vibrio cholerae*).
- He developed technique to strengthen immunity against anthrax bacteria by injecting weakened anthrax bacteria to healthy animal.
- Pasteur demonstrated a disease of silkworm was due to a protozoan parasite.

Contributions of Robert Koch

- He was a German microbiologist born on 11 December 1843.
- His contribution to the field of microbiology and medical science is the most valuable one.
- He developed for the first-time culture technique to culture the bacteria in the laboratory.
- He discovered bacteria caused tuberculosis of man.
- He developed for the first-time staining technique to stain the bacteria with acidic or basic stain.
- He isolated and identified different kinds of bacteria from various sample.
- He proved theory of germ diseases of Louis Pasteur by conducting investigative experiment.
- He was awarded Nobel Prize of medicine in 1905, formulating principles regarding diseases. These are now called „Koch Postulates".

Some of them are:-

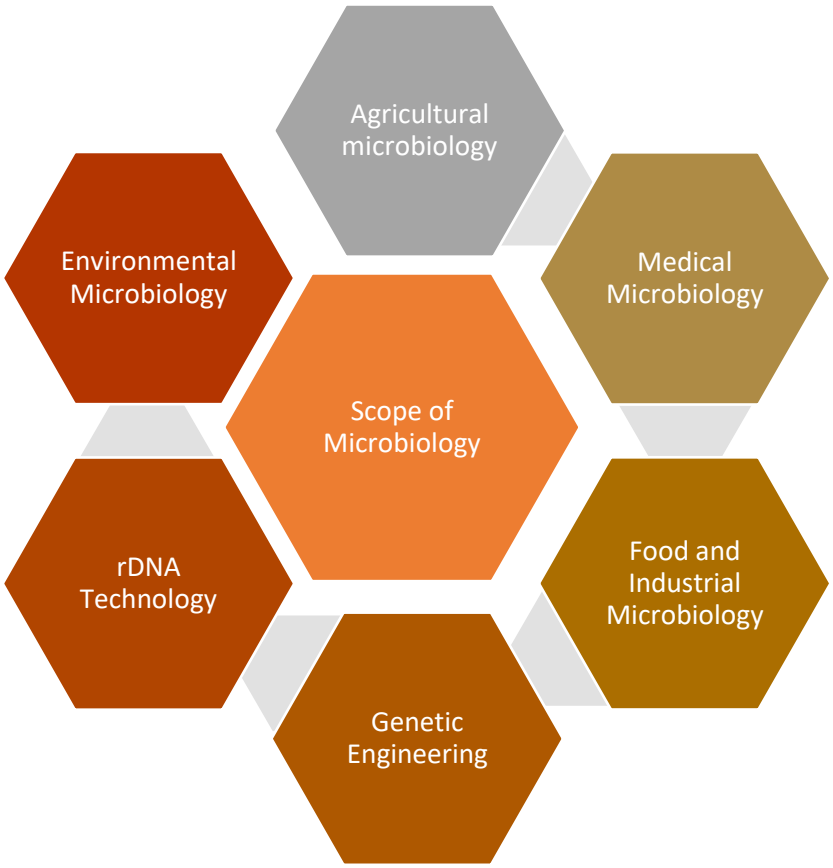
a. Specific pathogenic microbe causes one specific disease not more than one type of diseases in plants, animals and human beings.
b. Specific pathogenic microbes can be isolate from diseased organism and cultured outside the diseased organism.

Contributions of Alexander Fleming

- He was a Scotland doctor and biochemist born in 1881.
- He contributed knowledge about antibiotic Penicillin for this kind of work.
- He was awarded Nobel Prize in 1945.

His contributions to the field of microbiology can be summarized as below.

- He studied bacterial action in blood and their response to the antibiotic.
- He worked on antimicrobial substances. That is not toxic to human body but toxic to microbial body.
- He discovered bacteriolytic substance lysosome in the animal tissue.
- He developed technic to study sensitivity of the microbes to the antibiotic drugs.



Biogenesis Theory

Francesco Redi was an Italian physician, naturalist, and poet. • He is most well known for his series of experiments, published in 1668 as Experiments on the Generation of Insects, which is regarded as one of the first steps in refuting "spontaneous generation" - a theory also known as Aristotelian abiogenesis. At the time, prevailing wisdom was that maggots formed naturally from rotting meat.

Spontaneous Generation

The belief that life could originate from non-livingλ or decomposing matter The belief in the spontaneous generation of life from nonliving matter was introduced by **Aristotle,** who lived around 350 BC.

According to Aristotle, it was:

"readily observable that aphids arise from the dew which falls on plants, fleas from putrid matter, mice from dirty hay."

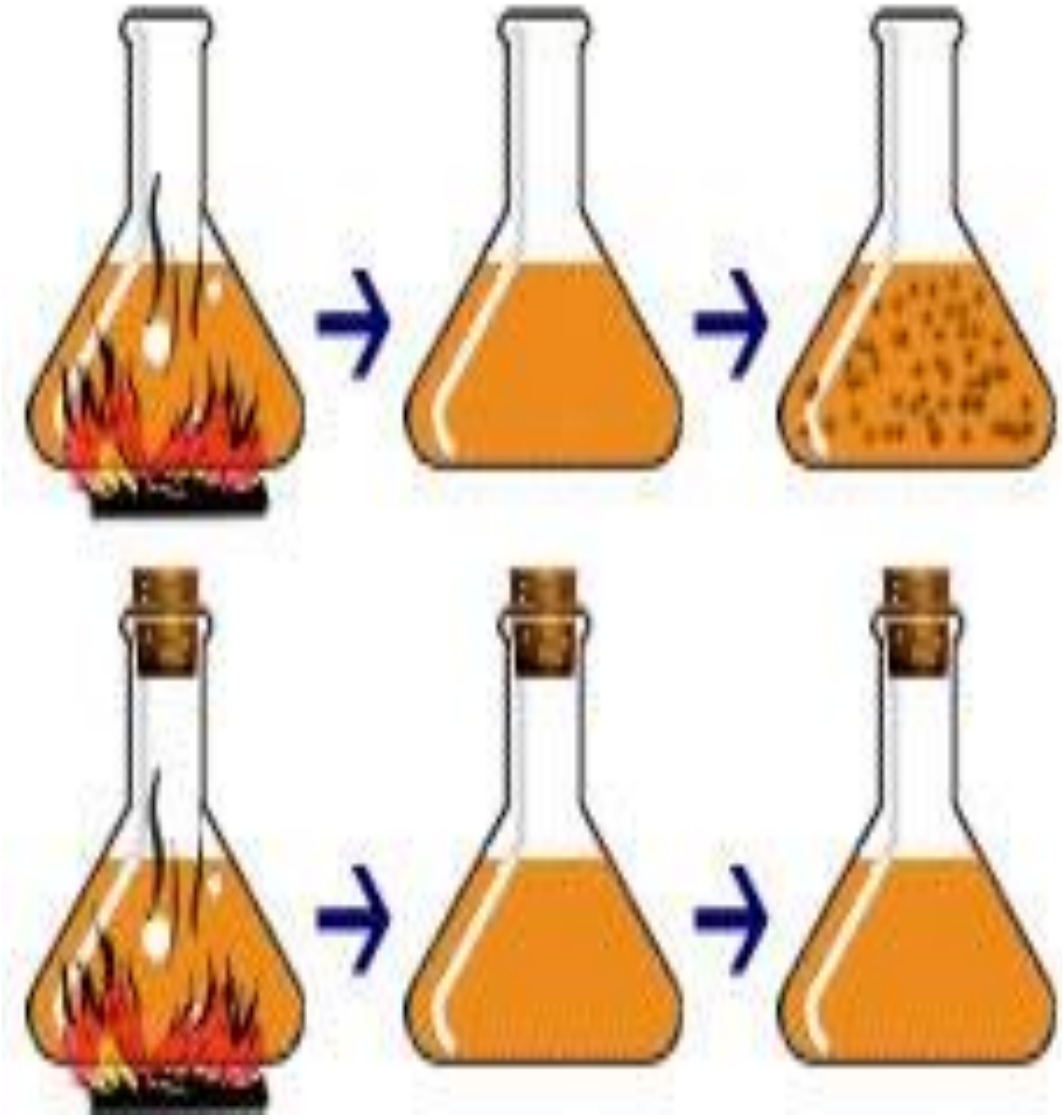
This belief remained unchallenged for more than 2000 years. The Theory of Spontaneous Generation was disproved and Microbiology was founded by some amazing scientists

This theory persisted into the seventeenth century, when scientists undertook additional experimentation to support or disprove it. By this time, the proponents of the theory cited how frogs simply seem to appear along the muddy banks of the Nile River in Egypt during the annual flooding. Others observed that mice simply appeared among grain stored in barns with thatched roofs. When the roof leaked and the grain molded, mice appeared. Jan Baptista **van Helmont**, a seventeenth century Flemish scientist, proposed that mice could arise from rags and wheat kernels left in an open container for 3 weeks. In reality, such habitats provided ideal food sources and shelter for mouse populations to flourish.

Lazzaro Spallanzani, an Italian abbot and biologist, tried several variations on Needham's soup experiments. First, he boiled soup for one hour, then sealed the glass flasks that contained it by melting the mouths of the flasks shut. Soup in those flasks stayed sterile. He then boiled another batch of soup for only a few minutes before sealing the flasks, and found that microorganisms grew in that soup. In a third batch, soup was boiled for an hour, but the flasks were sealed with real-cork corks (which, thus, were loose-fitting enough to let some air in), and microorganisms grew in that soup. Spallanzani concluded that while one hour of boiling would sterilize the soup, only a few minutes of boiling was not enough to kill any bacteria initially present, and the microorganisms in the flasks of spoiled soup had entered from the air.

- This initiated a heated argument between Needham and Spallanzani over sterilization (boiled broth in closed vs. open containers) as a way of refuting spontaneous generation. Needham claimed that

Spallanzani's "over-extensive" boiling used to sterilize the containers had killed the "life force." He felt that bacteria could not develop (by spontaneous generation) in the sealed containers because the life force could not get in, but in the open container, the broth rotted because it had access to fresh air, hence the life force inherent in its molecules, which contained and replenished the life force needed to trigger spontaneous generation. In the minimally-boiled flasks, he felt the boiling was not severe enough to destroy the life force, so bacteria were still able to develop

- 

Spallanzani was a Catholic who researched the theory about the spontaneous generation of cellular life in 1768. His experiment suggested that microbes move through the air and that they could be killed through boiling. His work paved the way for later research by Louis Pasteur, who defeated the theory of spontaneous generation. He also discovered and described animal (mammal) reproduction, showing that it requires both semen and an ovum. He was the first to perform in vitro fertilization, with frogs, and an artificial insemination, using a dog. Spallanzani showed that some animals, especially newts, can regenerate some parts of their body if injured or surgically removed. His great work, however, is the process of digestion., which he proved to be no mere mechanical process of trituration - that is, of grinding up the food - but one of actual chemical solution, taking place primarily in the stomach, by the action of the gastric juice. He also carried out important researches on fertilization in animals (1780).

– Needham: In return, Needham published a paper about the vegetative force, "the force that is the source of creation".

  He claimed that Spallanzani's experiments were invalid because boiling the juice for so long weakens the vegetative force

- **ANTONY VAN LEEUWENHOEK (1632-1723)** • He was the first Person, who invented the microscope and discovered the microbial world. • He was a draper (Merchant) from Delft, Holland. He used to grind lenses and made microscopes as a hobby. The microscopes of Leeuwenhoek could magnify objects about 200-300 times.
- • With his microscopes, Leeuwenhoek observed a variety of things like rain water, pond water and scrapings scrapings from his own teeth. He saw minute moving objects and called them as "Little animalcules", which we now know them as protozoa, yeasts and bacteria.
- • He made accurate sketches and communicated his findings to "Royal Society of London". • Thus, Leeuwenhoek was the first person to discover microscope and the presence of bacteria and spirochetes in mouth Leeuwenhoek discovered living cells while looking under his microscope.

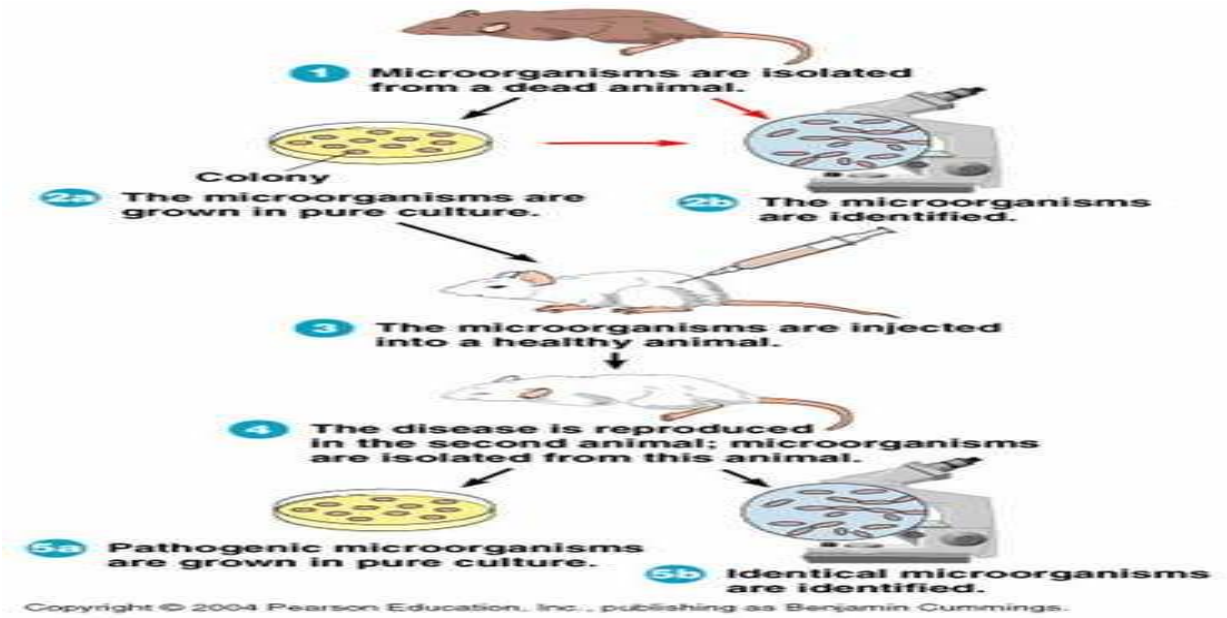
- He called the living cells, “Animalcules”.

  Animalcules were defined as tiny animals simple enough to be derived from non-living material

Louis Pasteur (1822 – 1895) French, is the Founder of Modern Microbiology 1861 Important contributions Disproves spontaneous generation theroy showed that yeasts converted sugar to ethanol and CO2 (1857) showed that sugar could be converted to lactic acid by certain animalcules (1857) showed that microorganisms were required to cause food spoilage showed that beer and wine were turned to vinegar in presence of microbes invented pasteurization Culture technique in liquid media Disease production by microbes Causative agents of Anthrax, Rabies Live attenuated vaccine, ARV

John Tyndall (1820-1893) – Omission of dust no growth. Demonstrated heat resistant◊ forms of bacteria (endospores

Prof. John Tyndall (1877) – demonstrated that dust carries microorganisms – showed that if dust was absent, nutrient broths remained remained sterile, sterile, even if directly directly exposed exposed to air – also provided evidence for the existence of exceptionally heat-resistant forms of bacteria



Robert Koch (1880s) – established the relationship between Bacillus anthracis and anthrax – used criteria criteria developed developed by his teacher teacher Jacob Henle (1809-1895) – these criteria now known as Koch's postulates • still used today to establish the link between a particular microorganism and a particular disease

• **Koch's postulates:** In 1890 the German physician and bacteriologist Robert Koch set out his celebrated criteria for judging whether a given bacteria is the cause of a given disease. Koch's criteria brought some much-needed scientific clarity to what was then a very confused field.

Koch's postulates are as follows:

- The bacteria must be present in every case of the disease.
- The bacteria must be isolated from the host with the disease and grown in pure culture.
- The specific disease must be reproduced when a pure culture of the bacteria is inoculated into a healthy susceptible host.
- The bacteria must be recoverable from the experimentally infected host.

However, Koch's postulates have their limitations and so may not always be the last word. They may not hold

- The particular bacteria (such as the one that causes leprosy) cannot be "grown in pure culture" in the laboratory.
- There is no animal model of infection with that particular bacteria.

A harmless bacteria may cause disease if:

- It has acquired extra virulence factors making it pathogenic.
- It gains access to deep tissues via trauma, surgery, an IV line, etc.
- It infects an immunocompromised patient.
- Not all people infected by a bacteria may develop disease-subclinical infection is usually more common than clinically obvious infection.

Despite such limitations, Koch's postulates are still a useful benchmark in judging whether there is a cause-and-effect relationship between a bacteria (or any other type of microorganism) and a clinical disease.

Alexander Fleming (1881 – 1955), a Scottish biologist and pharmacologist, observed bacterial staphylococci colonies disappearing on plates contaminated with mold.

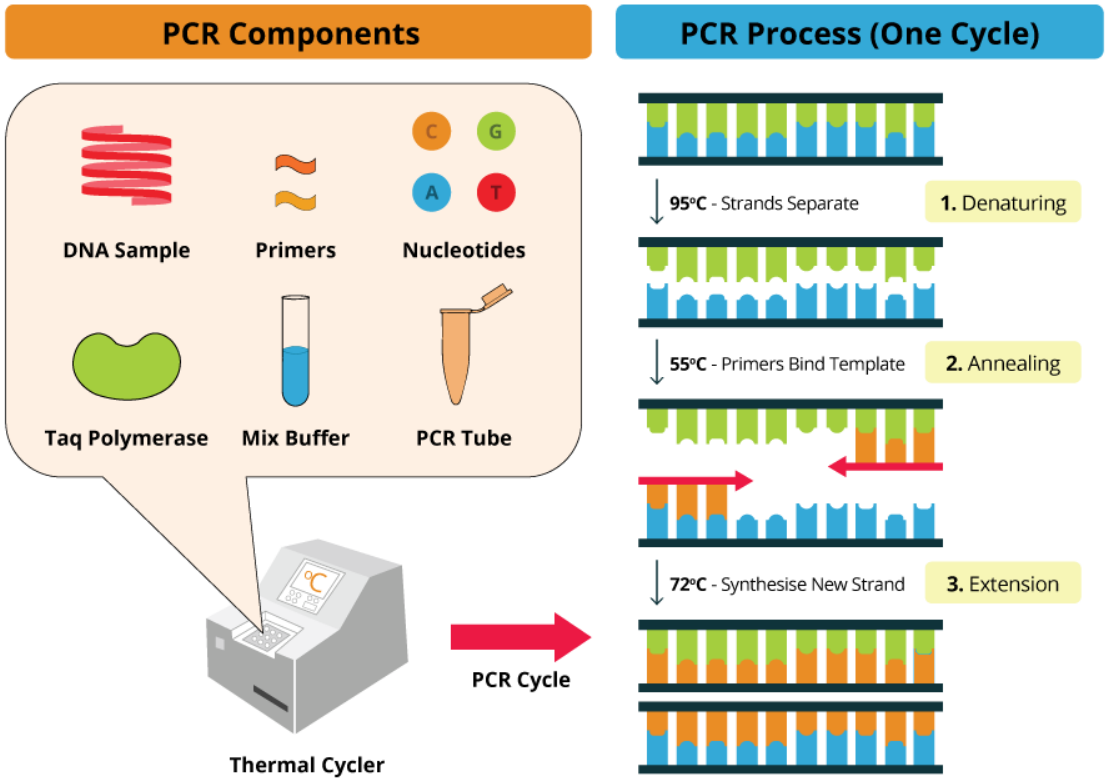
- Fleming extracted the compound from the mold responsible for destruction of the bacterial colonies.
- The product of the mold was named penicillin, after the *Penicillium* mold from which it was derived.
- Nobel Prize in Physiology of Medicine in 1945.

Contribution of Kary Mullis

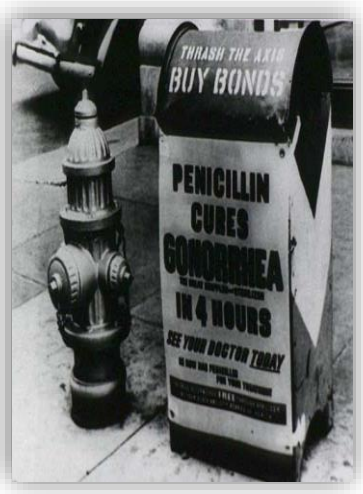
Kary Mullis developed PCR in 1983. He got nobel prize for Chemistry for his invention of the polymerase chain reaction (PCR), a simple technique that allows a specific stretch of DNA to be copied billions of times in a few hours.Earlier methods for obtaining a specific sequence of DNA in quantities sufficient for study were difficult, time-consuming, and expensive. PCR uses four ingredients: the double-stranded DNA segment to be copied, called the template DNA; two oligonucleotide primers (short segments of single-stranded DNA, each of which is complementary to a short sequence on one of the strands of the template DNA); nucleotides, the chemical building blocks that make up DNA; and a polymerase enzyme that copies the template DNA by joining the free nucleotides in the correct order.

These ingredients are heated, causing the template DNA to separate into two strands. The mixture is cooled, allowing the primers to attach themselves to the complementary sites on the template strands. The polymerase is then able to begin copying the template strands by adding nucleotides onto the end of the primers, producing two molecules of double-stranded DNA. Repeating this cycle increases the amount of DNA exponentially: some 30 cycles, each lasting only a few minutes, will produce more than a billion copies of the

original DNA sequence.



PCR has extremely wide applications. In medical diagnostics the technique made it possible to identify the causative agent of a bacterial or viral infection directly from a very small sample of genetic material; it was also used to screen patients for genetic disorders such as sickle cell anemia and Huntington's chorea. Evolutionary biologists employed PCR to study minute amounts of DNA extracted from the fossil remains of ancient species, and forensic scientists used it to identify crime suspects or victims from traces of blood, semen, or strands of hair left at a crime scene. The technique was also an important tool in gene sequencing.



Classification of microorganisms

The system of binomial nomenclature was introduced by Carl Linnaeus. Multiple local names make it extremely difficult to identify an organism globally and keep a track of the number of species. Thus, it creates a lot of confusion. To get rid of this confusion, a standard protocol came up. According to it, each and every organism would have one scientific name which would be used by everyone to identify an organism. This process of standardized naming is called as Binomial Nomenclature.

All living species including plants, animals, birds and also some microbes have their own scientific names. For eg.,

Rules of Binomial Nomenclature

A Biologist from all over the world follows a uniform set of principles for naming the organisms. There are two international codes which are agreed upon by all the biologists over the entire world for the naming protocol. They are:

- International Code of Botanical Nomenclature (ICBN) – Deals with the biological nomenclature for plants.
- International Code of Zoological Nomenclature (ICZN) – Deals with the biological nomenclature of animals.

These codes make sure that each organism gets a specific name and that name is globally identified.

The naming follows certain conventions. Each scientific name has two parts:

- Generic name
- Specific epithet

The rest of the **binomial nomenclature rules** for writing the scientific names of organisms include the following:

1. All the scientific names of organisms are usually Latin. Hence, they are written in italics.
2. There exist two parts of a name. The first word identifies the genus and the second word identifies the species.
3. When the names are handwritten, they are underlined or italicized if typed. This is done to specify its Latin origin.
4. The name of the genus starts with a capital letter and the name of the species starts with a small letter.

Why is Binomial Nomenclature Important?

As stated previously, there are millions of species of organisms distributed throughout the world. Furthermore, the same organisms are known by different names around the world and this can cause confusion when trying to identify or classify. Hence, binomial nomenclature was seen as a viable solution to this problem.

Drawbacks of Binomial Nomenclature

Some of the basic drawbacks of binomial nomenclature are:

- If two or more names are currently in use, according to the law of priority, the correct name will be the one used first and the others end up being synonyms as validity is the senior synonym. Providing stability in the naming and classification of organisms must be emphasized.
- Also, the names used prior to those included in the "Systema Naturae", by Linnaeus are not recognized.

FIVE KINGDOMS AND THE 3 DOMAIN CLASSIFICATIONS OF MICROORGANISMS

Classification: classification is a scheme by which various organisms are arranged according to the relationship between the individuals and groups.

In 1969, R. H. Whittaker proposed a five kingdom classification scheme that has been widely accepted universally. These five kingdoms are

- Monera
- Protista

- Fungi
- Plantae
- Animalia

Viruses are non-cellular molecular particles that remain on the threshold of life between living and non-living viruses are not included in any of these kingdoms and are treated as a separate group.

FIVE KINGDOMS

I. KINGDOM: MONERA (PROKARYOTA)

It includes two major groups namely bacteria and cyanobacteria blue green algae.

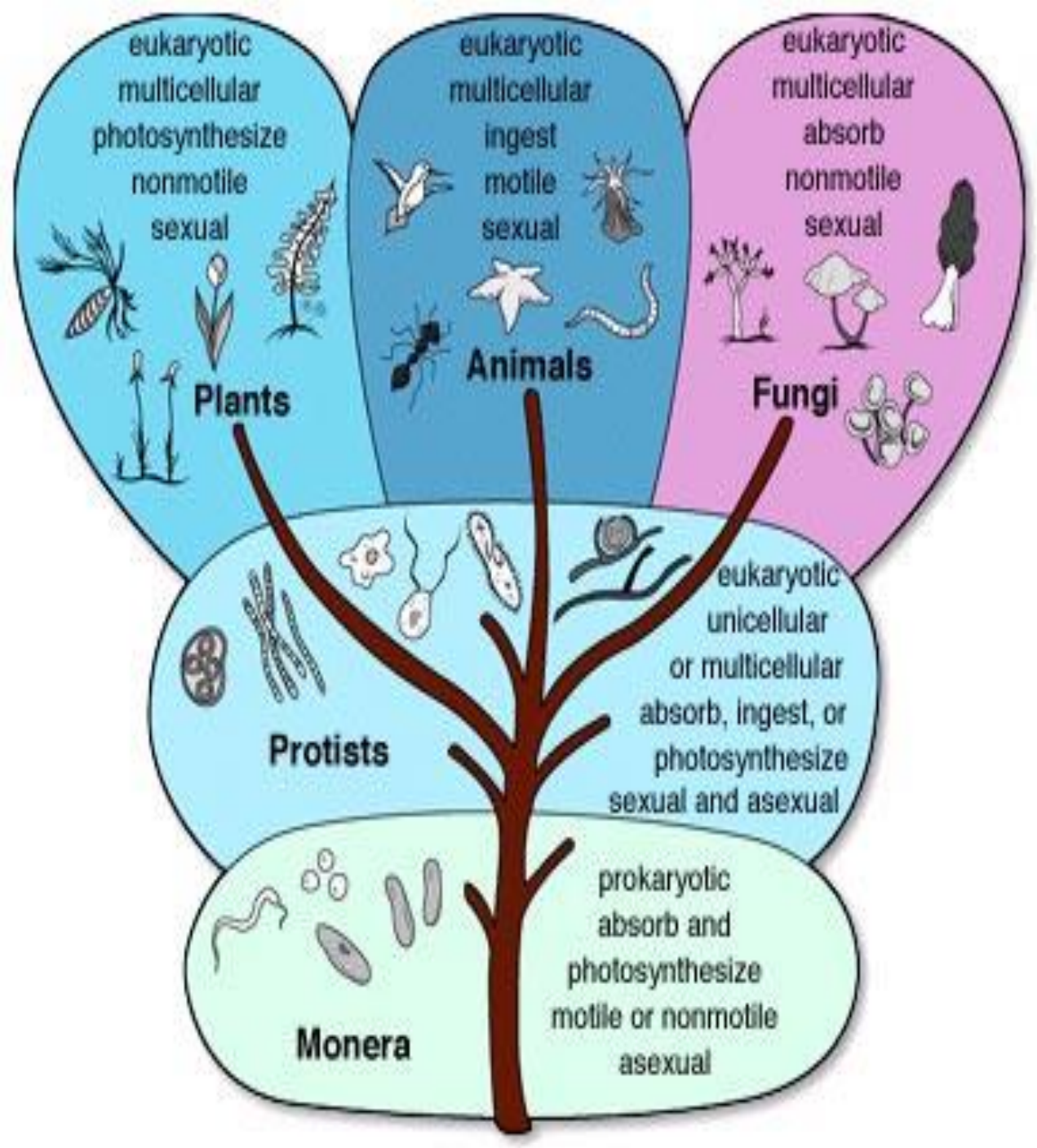
Salient features of Monera

- Monerans are present in both living and non-living environment.
- Some have rigid cell walls, while some do not.

Five Kingdom classification

- Membrane bound nucleus is absent in monerans.
- Habitat – Monerans are found everywhere in hot or thermal springs, in the deep ocean floor, under ice, in deserts and on or inside the body of plants and animals.
- They are autotrophic, i.e., they can synthesize food on their own while some others have a heterotrophic, saprophytic, parasitic, symbiotic, commensalistic and mutualistic modes of nutrition.
- Locomotion is with the help of flagella.
- Circulation is through diffusion.
- Respiration in these organisms vary, few are obligate aerobes, while some are obligate anaerobes and facultative anaerobes
- Reproduction is mostly asexual and few also reproduce by sexual reproduction.

- Usually the cells undergo reproduction by budding or binary fission.
- Examples: *Mycobacterium, E.coli, Strepto coccus*



Kingdom Protista

The term „Protista" is derived from the Greek word "*Protistos*", meaning "*the very first*".

These organisms are usually unicellular and the cells of these organisms contain a nucleus which is bound to the organelles. Some of them even possess structures that aid locomotion like flagella or cilia.

Salient features

- They are simple, unicellular, eukaryotic organisms.
- Most of the protists live in water, some in moist soil or even the body of human and plants.
- These organisms have a membrane-bound nucleus, endomembrane systems, mitochondria for cellular respiration and some have chloroplasts for photosynthesis.
- Nuclei contain multiple DNA strands and the number of nucleotides is significantly less.
- Respiration – cellular respiration is the primarily aerobic process, but some living in the moist soil underneath ponds or in digestive tracts of animals are facultative anaerobes.
- Locomotion is often by flagella or cilia.
- Nutrition- include both heterotrophic and autotrophic.

- Reproduction – Some reproduce sexually and others asexually.
- Some protists are pathogens of both plants and animals. Example: Plasmodium falciparum causes malaria in humans.
- Examples: *Amoeba, Paramecium, Euglena.*

Kingdom Mycota (Fungi)

These include yeast and molds. These are non-photosynthetic heterotrophs having either parasitic or saprophytic mode of nutrition.

General features of fungi are as follows:

- Fungi are eukaryotic, non-vascular and non-motile organisms.
- The growth rate of fungi is slower than that of bacteria.
- The Kingdom Fungi grow best in an acidic environment.
- The Kingdom Fungi consist of both unicellular (e.g. Yeast, Molds) and multicellular (e.g. mushrooms) organisms.
- Like plant cells, fungi have cell walls made up of complex sugar molecules called chitin. But unlike plants, they do not undergo photosynthesis.
- The vegetative body of the fungi may be unicellular or composed of microscopic threads called hyphae.
- They have a heterotrophic mode of nutrition. Few species are saprophytes i.e., they feed on dead and decaying organic matters.
- Some fungi are parasitic while some are symbionts.
- Reproduction in fungi is both by sexual and asexual means.
- Examples: *Mycorrhiza, Saccharomyces* etc.

Kingdom Plantae

- Plants are multicellular organisms compared of eukaryotic cells.
- The cells are organized into tissues and have cell wall.
- They obtain nutrients by photosynthesis and absorption.
- They are primarily non-motile and live anchored to a substance.
- Reproduction is sexual and asexual.

- Ex: mosses, ferns, conifers and flowering plants.

Kingdom Animalia

Animals are multicellular organisms composed of eukaryotic cells.

- The cells are organized into tissue and lack cell wall.
- They do not carry out photosynthesis and obtain nutrients primarily by ingestion.
- Many animals are adapted for locomotion.
- Heterotrophic mode of nutrition.
- They reproduce by sexual mode of reproduction.
- Ex: sponges, worms, insects and vertebrates.

THREE DOMAIN CLASSIFICATIONS

This system was proposed by *Carl Woese* in 1978 on the basis of molecular biology and biochemistry. This classification is entirely dependent on the differences in the nucleotides sequences of rRNA in the cells and also differences in cell membrane lipids structure. The sequence similarity in the rRNA molecule provided a strong basement to predict the evolutionary classification of microbes.

According to this classification system and ancestor cell give rise to three different cell types. Each representing a domain viz; Archaea, the Bacteria (prokaryotes) and Eukarya (eukaryotes) which includes algae, fungi Protozoa, plants and animals.

I. The Archaea (archaebacteria)

Archaea bacteria represent a unique group of microorganisms that are related to bacteria, but might have deviated from the evolutionary live of bacteria very early during the evolution of Monera. They are considered as the primitive bacteria.

Salient features of Archaea

1. The cell wall lack (peptidoglycan) (psedopeptidoglycan).

2. The membrane consist of characteristic lipids i.e. the lipids have branched hydrocarbon that increase the fluidity of the membrane.

   In some Archaea bacteria the plasma membrane is a monolayer composed of glycerol tetra ether lipids.

- The genome consists of single covalently closed circular DNA.

Summary

- Some of the Archaea bacteria can survive in extreme environment such as high temperature (Thermophiles) extremely halophilic (Salt Lakes, tidal pools) and anaerobic environments (methanogenic bacteria).
- The archaea are insensitive to certain antibiotics (ex: chloramphenicol) but are sensitive to diphtheria toxin.
- They are unicellular prokaryotes.
- The bacterial cell wall contains peptidoglycan (murein).
- The cell membrane is composed of phospholipids.
- Bacteria are sensitive to some common antibiotics like tetracycline, ampicillin, and penicillin.
- The cytoplasm contains double stranded covalently closed circular DNA.
- Bacteria contain rRNA that is unique to the bacteria, as indicated by the presence of molecular region distinctly different from the rRNA of archaea and eukarya.
- Bacteria include mycoplasmas, cyanobacteria, gram positive and gram negative bacteria.

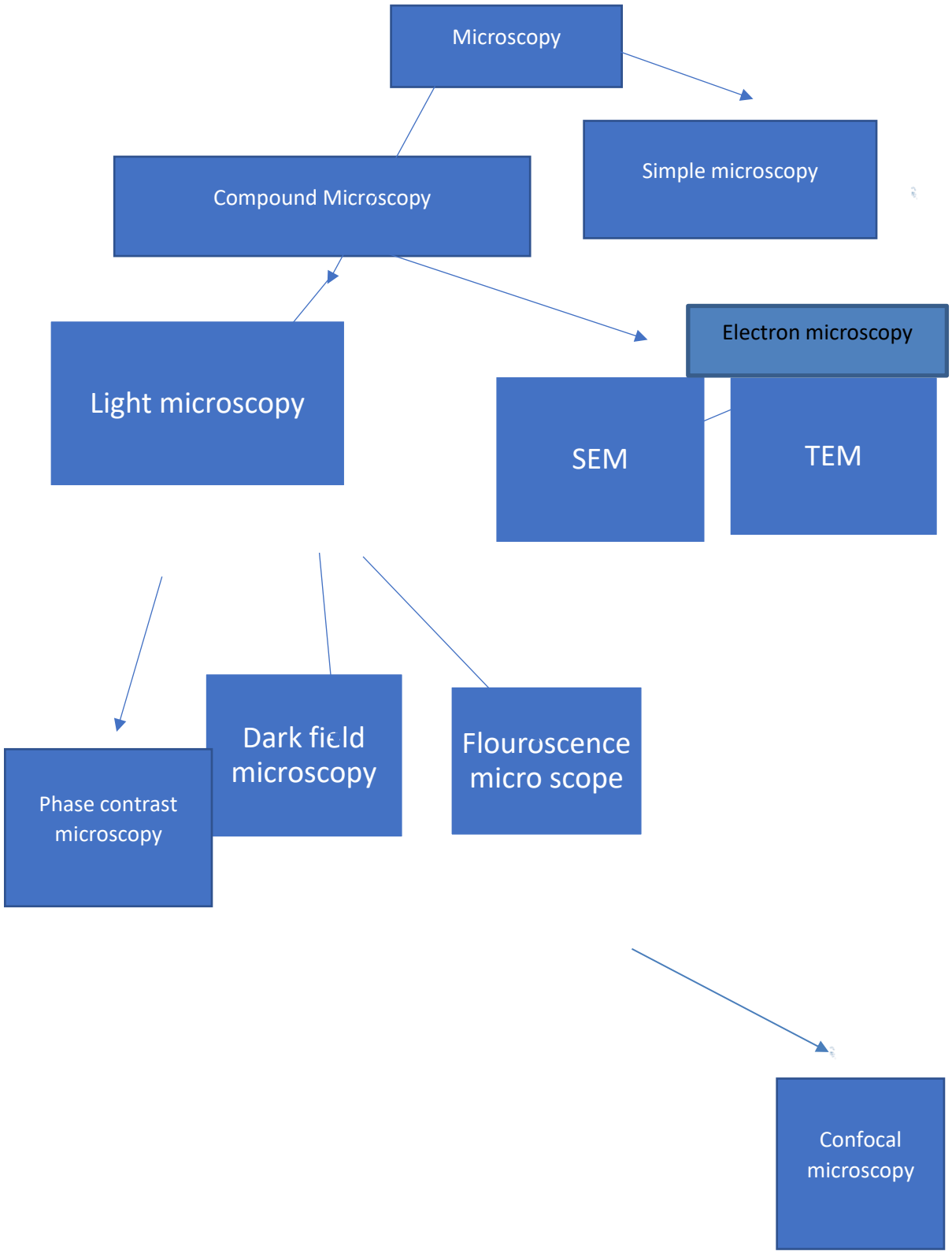
The eukarya (also called as eukarya) possess the following characteristics.

Eukarya have eukaryotic cells.

- Like the bacteria, they have membranes composed of unbranched fatty acid chains attaches to glycerol by eater linkage.
- Not all eukarya possess cells which a cell wall, but for those eukarya having a cell wall that wall contains no peptidoglycol.
- Eukarya contain rRNA that is unique to the eukarya as indicated by the presence of molecular regions distinctly different from the rRNA of Archaea and Bacteria.

Questions

1. Describe Abiogenesis
2. Explain Spontaneous generation of life
3. Define Pasteurization
4. Define Germ theory
5. State Koch's postulate
6. List recent development in the field of microbiology
7. Givet the contributions of
8. Louis Pasteur Anton von Leewenhoeck
9. Robrt Koch
10. Alexander Flemming
11. Karl Mulis
12. Joseph lister
13. Explai classification of microorgamisms
14. Five kingdom Classification
15. Three kingdom classification


Microscopy
Simple microscopy
Compound Microscopy
Light microscopy
Electron microscopy
SEM
TEM
Phase contrast microscopy
Dark field microscopy
Flouroscence micro scope
Confocal microscopy

UNIT-II

MICROSCOPY

Microscope is an instrument used to observe the objects which are not visible to our naked eye. Faber (1625) used the term microscope and it is derived from two Greek words namely, Mikros= Small ; Skopian= To See.

Z. Janssen (1590) invented simple microscope composed of two lenses to magnify the smaller abject. Antony Van Leeuwenhoek (1667) who was wrongly credited as inventor of compound microscope actually invented simple microscope by using convex lenses of high magnification power up to 300x. Robert Hooke (1668) invented compound microscope with two kinds of magnifying lens systems namely objective lens system and ocular lens system.

Types of Microscopes:

Basically microscopes are classified into two main types namely:

1. Light microscopes
2. 2. Electron microscope

1. **LIGHT MICROSCOPES:** In these, light rays constitute the source of illumination to focus the object or specimen. These include;

a. Simple microscope- It contains single lens system to magnify the specimen.

b. Compound microscope- It contains two lens systems to magnify the specimen.

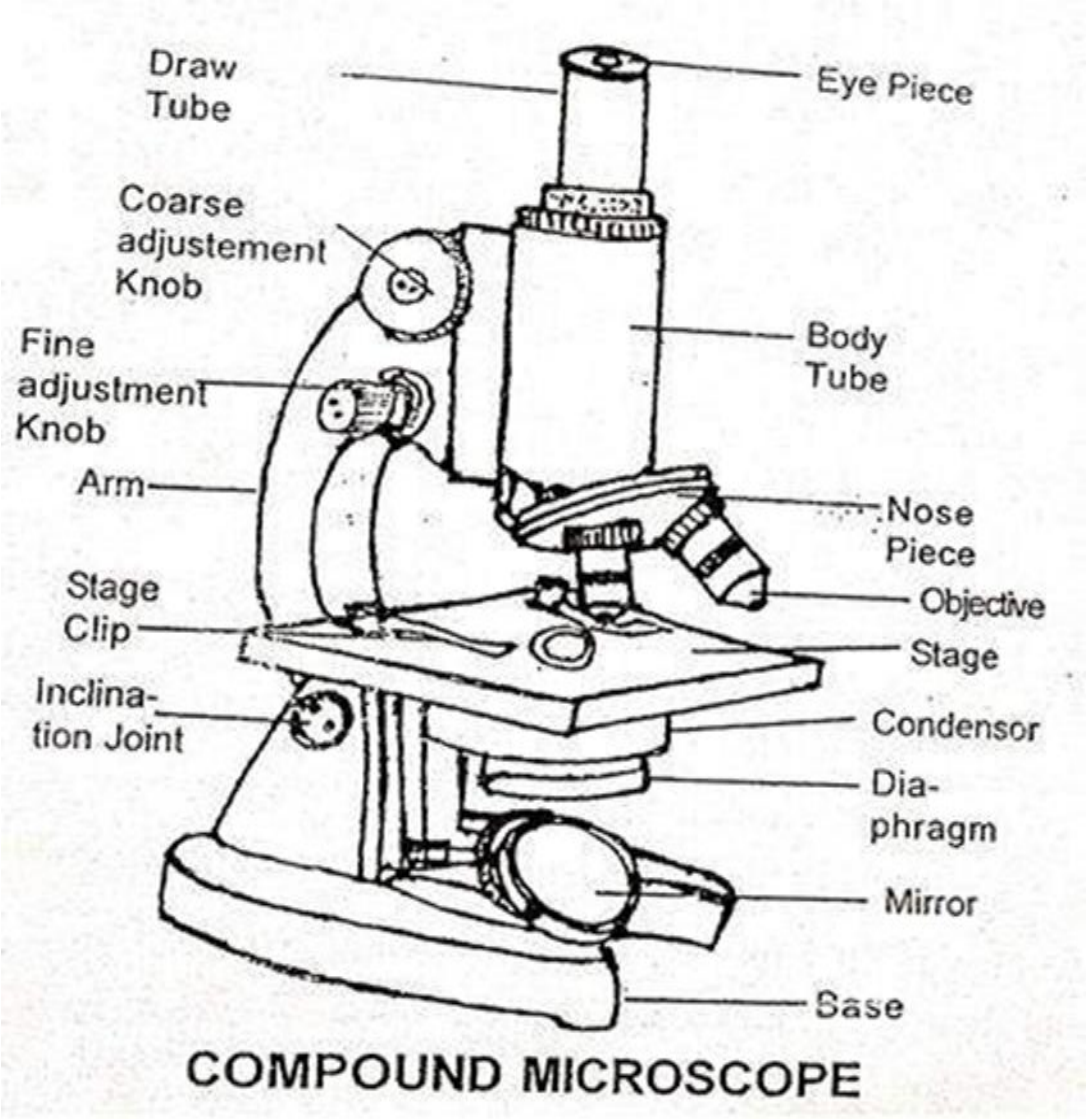
2. **ELECTRON MICROSCOPES:** In these Electron beam constitute the source of illumination.

Light microscopes are of following types:

1. Bright field microscope
2. Dark field microscope
3. Fluorescence microscope
4. Phase contrast microscope

5. Ultraviolet microscope

6. Interference microscope



COMPOUND MICROSCOPE

It is an optical instrument used to observe the specimen, which is not visible to our naked eyes.

Parts of microscope

- *Eye piece*: it is placed on top of the draw tube of the microscope. It contains two plano convex lenses of which upper smaller one is eye piece and lower larger is field lenses. Eye piece magnifies primary image of the specimen and produces is secondary image.
- *Body tube*: it is cylindrical tube with upper narrow draw tube. It holds eye piece at one end and

objectives at other end at the proper working distance.

- *Coarse adjustment*: it helps to move the body tube up and down to get rough focusing of the specimen.
- *Fine adjustment*: it also helps to move body tube up and down, but very slightly. It helps to get fine adjusting (focusing) of the specimen.
- *Arm*: it holds body tube coarse adjustment and fine adjustment.
- *Nose piece*: it is fixed to the lower end of the body tube. It holds objective of different magnifying powers, like, 10X, 45X and 100X. it permits the interchange of objectives from low

- power to high power and vice versa.
- *Objectives*: these contains plano-convex lense of different magnifying power, where magnifying power of low power the objective is low, high power objectives is 45X and oil immersion objective is 100X, objective magnifies specimen and produces its magnified primary image.
- *Stage*: it provides the place for the specimen slide over whole image in it.
- *Stage clip*: this helps in firm attachment of specimen slide on the stage.
- *Condenser*: it is a large plano-convex lens placed below the hole of the stage. It collects, condenses and focus the light rays in the focus of thick beam on the specimen.
- *Diaphragm*: it is placed below the condenser, it helps to relate amount of light rays which are pairing through it.
- *Mirror*: it reflects the light rays through the diaphragm. Condenser and hole is the stage concave mirror is used to focus the natural light rays, plane mirror is used to focus the electric light rays.
- *Base/foot*: it bears complete weight of the microscope and gives support to it.

Operation of the compound microscope to observe specimen slide:

- Bring microscope to the normal vertical position of the microscope is tilted.
- Fix the lower objective (10X) of the microscope to its proper position by operating nose piece.

- Keep the diaphragm is fully open condition by operating the node of the diaphragm.
- Adjust the concave mirror of the microscope is such a way that bright light can be seen through the eye piece.
- Keep specimen slide on the stage over the whole in it.
- Bring the lower power objective near to the specimen slide by operating coarse adjustment or fine adjustment.
- After focusing the specimen slide to this stage with the help of stage clips.

DARK FIELD MICROSCOPE (DFMS):

It is a compound microscope with optical lens units in which microscopic field is dark, while object appears bright.

In the dark field microscopy, the microbes are observed as bright objects against a dark background. This is achieved by fitting a special kind of condenser (Abbe's condenser) with an opaque disk that can direct the light path from the source of illumination. In the dark field microscope a special condenser fits in to the sub stage in place of the ordinary condenser.

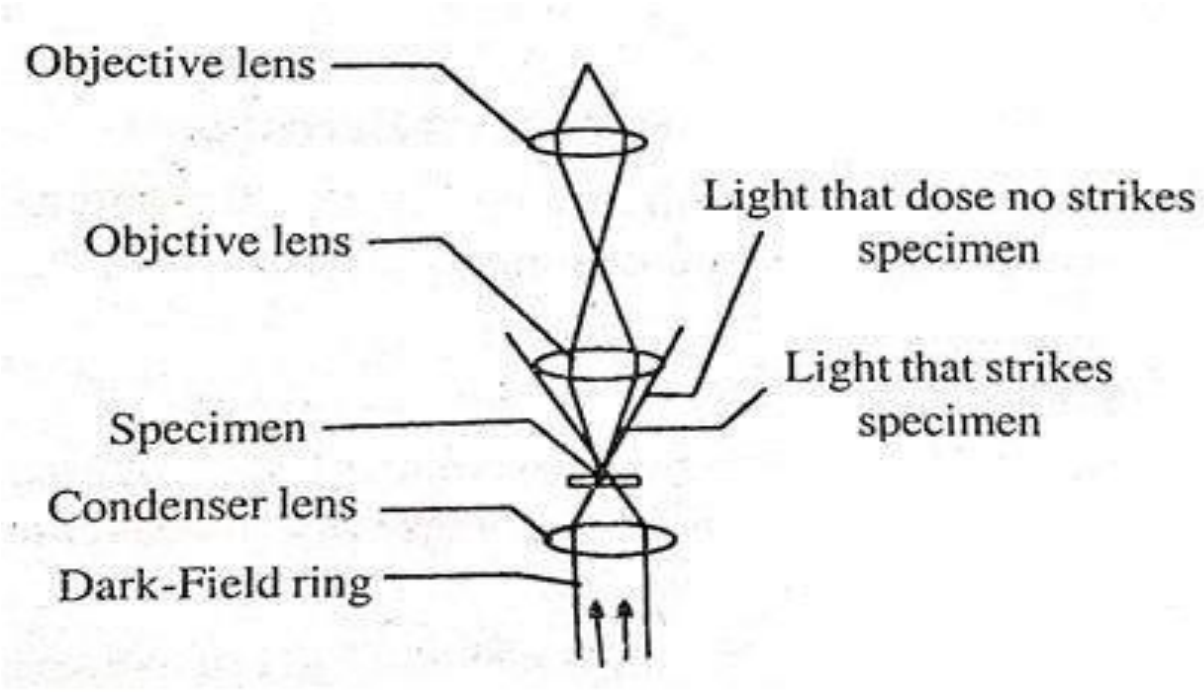
The central of this special condenser is opaque, so none of the central light rays can pass through it, and the object is illuminated only with very oblique rays, which are almost parallel to the stage.

Most of the light directed through the condenser does not enter the objective thus the field is essentially dark. However if transparent medium contain objects (microbes/cells) that differ in their refractive index, there will be scattering of light by reflection and retraction.

The scattered light will enter the objective and thus the object will appear bright in an otherwise dark field. For low power magnification we usually employ Abbe condenser but, for higher magnification additional condensers such as carboid and paraboloid are employed with other objectives.

Dark field microscopy is used for the examination of live, unstained preparations of microbes or other specimen suspended in fluid and is useful for diagnosis of disease. The bacterial motility can be studied

by using dark field microscope. It is especially useful for the study of very small and delicate organisms such as spirochetes.



Dark field Microscopy

Uses: It is used to observe living unstained specimen particularly those which stain poorly.

PHASE CONTRAST MICROSCOPE (PCM)

It is useful for visualizing internal structure of transparent living unstained cells. This microscope was originally developed by **Zernike** in 1935, hence called *Zernike microscope*. Zernike awarded Nobel Prize in the year 1953 for the discovery of principle behind phase contrast microscope.

The construction of this microscope is based on the principle that through biological specimens are highly transparent to visible light, they cause phase transitions in the transmitted radiations. These differences that results from small differences in the refractive index and / or thickness of different parts

of object can be made clearly detectable with this microscope.

Structural Features:

PCM in its structural features is similar to that of bright field compound microscope but with the following 2 additional parts:

1. *Annular diaphragm (AD):* It has circular opening to allow hollow cone of light and placed in the place of normal diaphragm.

2. *Phase shifting plate (PSP):* It is a thick circular glass plate with thin annular region, which coincides with hollow cone of light coming through the annular diaphragm. It is placed above the upper lens in the objective.

Working Principle

When the light rays of same frequency passes through transparent object (glass plate) having thicker and thinner regions, they refract at different degrees and results different phase of light, where light rays travels slowly through the thicker region of the glass plate and light rays travels fast through the thinner region of glass plate. This is the principle involved in the working of phase contrast microscope (PCM).

Similarly when the light rays passes through the different components of the cell such as nucleus, mitochondria, chloroplast etc. having different thickness, they refract at different rates. These results in different phases of light, phase contrast microscope convert all such different phase of light into visible variations. Hence different components of the cell can be seen when the cell is observed under PCM.

Working method

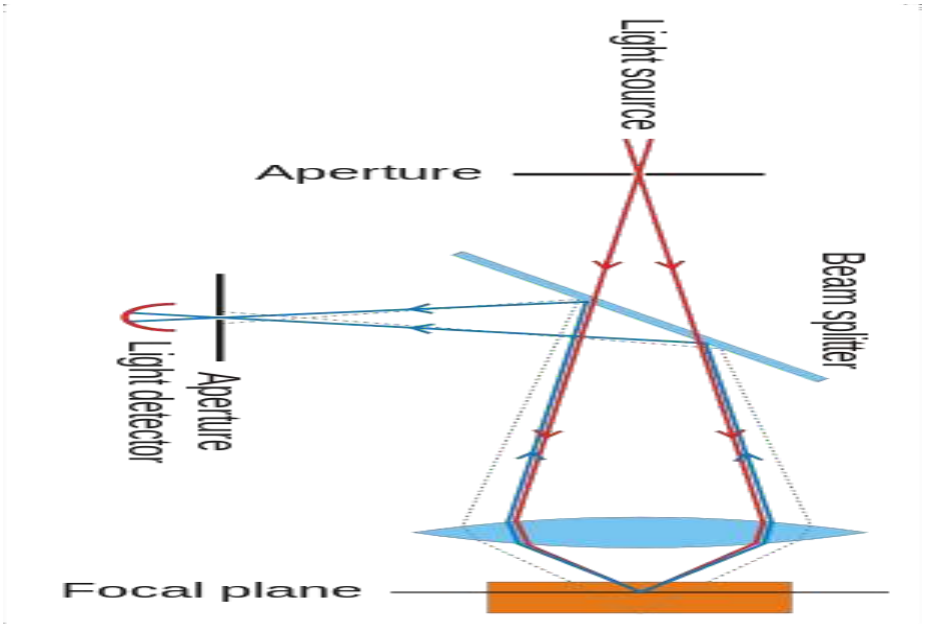
When hollow cone of light is focused on the specimen through the annular diaphragm, light rays at the point of specimen split into direct rays and refracted rays. Direct rays pass through the objective lens and thinner annular region of PSP. Refracted rays by the cellular objects passes through the objective lens and thicker region of PSP.

Direct rays are more advanced over refracted rays but with a little variation. PSP convert such little variations in phases of light into visible variations. As a result of this different parts of the cell show different brightness and darkness in the final image

Uses: PCM is used to observe different components like nucleus, mitochondria, chloroplast etc. in unstained living cells,

CONFOCAL MICROSCOPY

An optical imaging technique for increasing optical resolution and contrast of a micrograph. Radiations



emitted from laser cause sample to fluoresce. Uses pinhole screen to reduce high resolution images. Eliminates out of focus, So images have better contrast and are less hazy. A series of thin slices of the specimen are assembled to generate a 3-dimensional image. Is an updated version of fluorescence microscopy.

Principle:

In confocal microscopy two principles are typically used:

1. A pinhole is placed in front of the illumination source to allow transmission only through a small area.
2. This illumination pinhole is imaged onto the focal plane of the specimen. i.e. only a point of the specimen is illuminated at one time.
3. Fluorescence excited within the focal plane of the specimen will go through the detector pinhole.
4. Scanning of small sections is done and joined them together for better view.

Working mechanism:

Confocal microscope incorporates 2 ideas:

1. Point-by-point illumination of the specimen.
2. Rejection of out of focus of light.

- Laser provides intense blue excitation light.
- The light reflects off a dichroic mirror, which directs it to an assembly of vertically and horizontally scanning mirrors.
- These motor driven mirrors scan the laser beam across the specimen.
- The specimen is scanned by moving the stage back and forth in the vertical and horizontal directions and optics are kept stationary.
- Dye in the specimen is excited by the laser light and fluoresces.
- The florescent (green) light is descanned by the same mirrors that are used to scan the excitation (blue) light from the laser beam.
- Then it passes through the dichroic mirror.

- Then it is focused on the pinhole.
- The light passing through the pinhole is measured by the detector such as photomultiplier tube.
- For visualization detector is attached to the computer, which builds up the image at the rate of 0.1-1 second for single image.

Applications:

1. Confocal microscopy allows analysis of florescent labeled thick specimens without physical sectioning.
2. Three dimension reconstruction of specimen.
3. More colour possibilities- because the images are detected by a computer rather than by eye, it is possible to detect more colour differences.
4. Improved resolution.

FLUORESCENCE MICROSCOPE (FMS):

It is an ordinary compound microscope with the two structural features like special type of filters and UV light rays transmitting condenser. Since UV light rays will be the source of illumination for this microscope, it is also called **UV Microscope**.

In this microscope invisible UV rays are converted into visible fluorescent rays, hence it is named as Fluorescence Microscope.

Structural features

Structural features of fluorescence microscope are similar to that of bright field microscope except for the following:

In Fluorescence microscope

- UV rays transmitting condenser is used in the place of normal condenser.

- two special kinds of filters are used, they are

a. *Excitation filter or Primary filter* - This is placed in between specimen side and condenser just below the stage. This filter allows only UV rays and light rays of shorter wavelength to pass through it.

b. *Barrier filter or Secondary filter* - This is placed in between two lenses of eyepiece. This filter acts as barrier for the UV rays and allows only fluorescent light rays.

Working Principle:

Certain chemical dyes when exposed to UV rays, they absorb invisible UV rays and emit them in the form of visible fluorescent light rays. This is the principle involved in this microscope.

Regarding the principle involved in the magnification of the specimen, it is similar to that of Bright field microscope.

Working method:

1. The bacterial cells which are to be observed under the fluorescent microscope are to be stained with fluorescent dye like acridine.
2. When such slide is observed under the microscope, fluorescent dye of the bacterial cells absorb UV light rays coming from UV rays transmitting condenser and emit them in the form of visible fluorescent rays. As a result of this bacterial cells are made visible better than that of normal stained bacterial cells.

Uses: Fluorescence microscope is used to get structural details and biochemical events going on in the bacterial cells, whereas this is not possible with normal microscope.

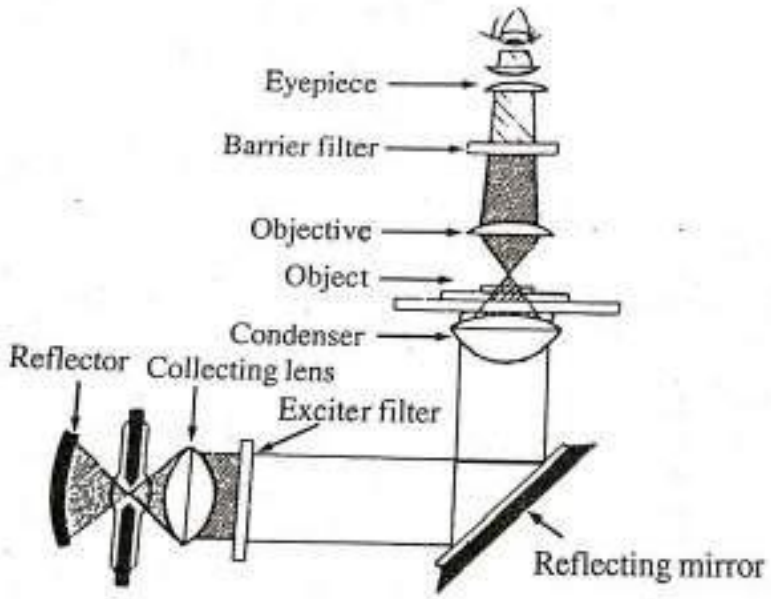


Diagram to illustrate the component parts of the fluorescent microscope.

ELECTRON MICROSCOPE:

It is a magnifying instrument in which electron beam constitute the source of illumination.

The wavelength of the electron beam is 0.5mμ, hence it is possible to resolve the object even as small as 1mμ (10Å) size. In compound microscope it is not possible to resolve the object having size less than 0.1 micron (100mμ).

Resolving power of electron microscope is 200 times more than that of compound microscope, where it magnifies the object 1,00,000 times or more of its original size.

Principle of Working

In the electron microscope, the source of illumination is the beam of electrons. In order to understand the working principle of electroscope, one must first understand some elementary properties of electrons.

The electrons are negatively charged sub atomic particles that around the atomic nucleus at high velocity in specific electron orbits.

In 1924 De Broglie working on electrons proposed the dual nature of electrons i.e., electrons can behave both as a particular as well as waves and should have both a fixed wavelength and frequency. The wavelength of the electrons is calculated by De Broglie's formula.

$$\lambda = \frac{h}{mv}$$

v → velocity
m → mass
h → plank's constant

Electron microscopes are of 2 basic designs:

1. Transmission electron microscope (TEM)
2. Scanning electron microscope (SEM)

TRANSMISSION ELECTRON MICROSCOPE (TEM)

M. Ruska first developed TEM in 1931, for which he was awarded Nobel Prize in 1986. In TEM Electrons are made to transmit through the specimen to produce the magnified image/ photograph of the specimen.

TEM has resolution limit 10Å and resolution power 400 to 2000 times more than that of compound microscope.

Structural features of TEM: it consists of the following units:

1. Electron gun: it consists of Tungsten filament. This filament when heated emits elctro-magnetic rays (electron).

2. Electromagnetic condenser: it condenses electromagnetic rays into a thick electron beam.
3. Perforated metallic grid: it provides the place to keep the specimen to be observed under the microscope. Fluorescent screen: it traps the final magnified image of the specimen. Above parts are assembled in a vacuum chamber as one unit.

Working principle: it is similar to that of principle involved in working of light microscope, but here electron beam is used as the source of illumination. Electromagnetic condenser is used to condense electromagnetic radiations.

Electromagnetic objective and electromagnetic ocular are used as magnifying units. Electromagnetic rays when passes through the specimen and electromagnetic objective, magnified primary image of the specimen will be obtained.

When electromagnetic rays further passes through the electromagnetic ocular, highly magnified secondary image of the specimen will be obtained.

Working method:

Preparation of the specimen: the specimen which is to be observed under TEM is subjected to dehydration, freezing, ultra sectioning and staining with metals of high atomic weight like gold, platinum, uranium. Then the specimen is placed on the perforated metallic grid of TEM.

Focusing of the specimen: when tungsten filament is heated in the electron gun, it emits electron. These electrons are allowed to pass through the electromagnetic condenser, specimen, electromagnetic objective and finally through the electromagnetic ocular to get the magnified final image of the specimen on the fluorescent screen. This image can also be recorded on photographic plate.

Uses:

- it is used to magnify the specimen having size between 1mμ to 100mμ.
- Used to study ultra-structure of the specimen.

Electromgnetic objective: it magnifies the specimen and produces final image

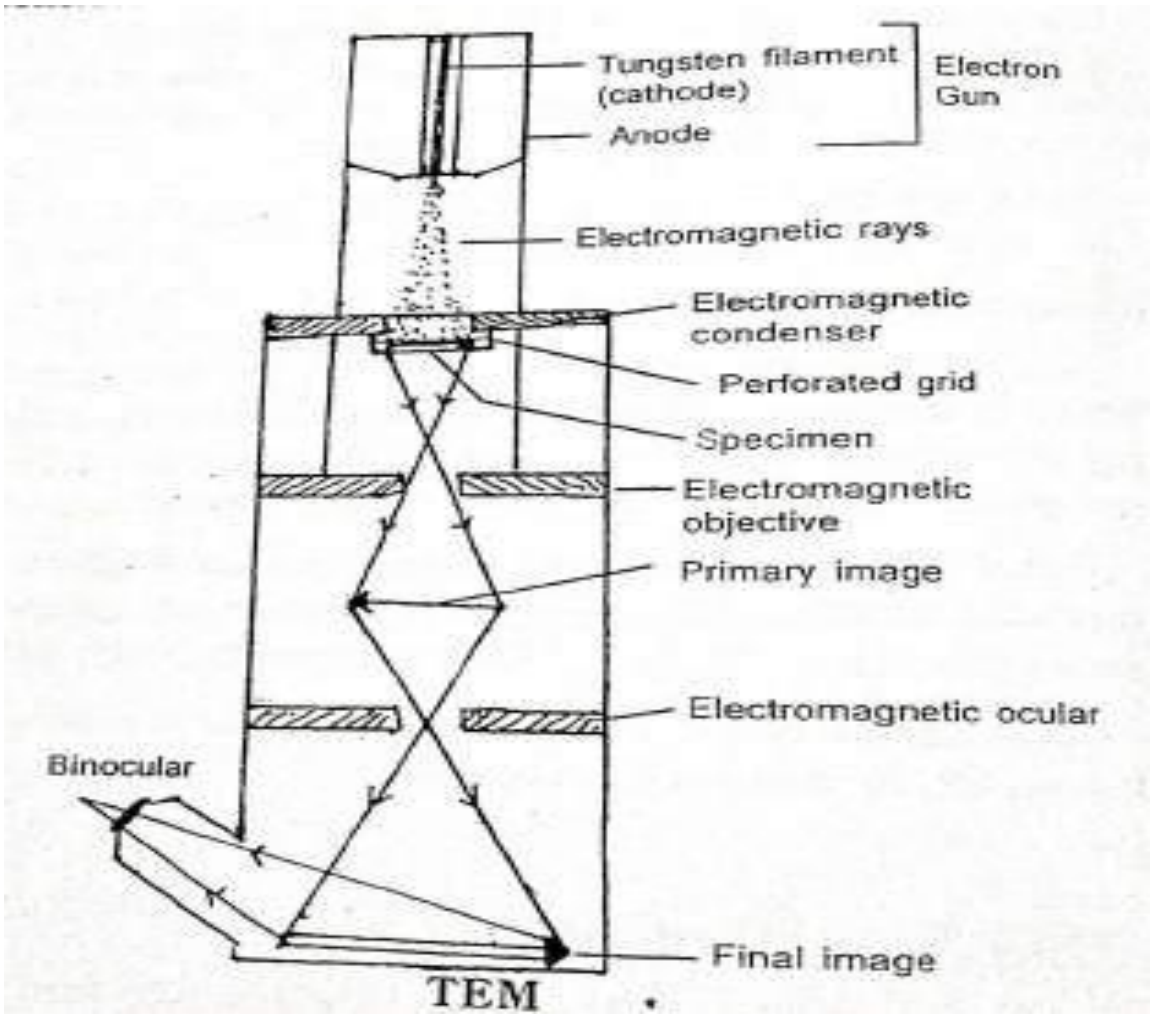
Scanning Electron Microscope (SEM)

SEM

Dennis Mc. Mullan first developed SEM in 1948. In this microscope electrons are reflected from the specimen instead of passing through it, hence electrons scan only surface details of the specimen. Resolving power and resolution limit of this microscope is less than TEM.

Working principle: it is same as that involves in TEM, but unlike TEM, here electrons scan the surface details of the specimen.

Working method: specimen which is to be observed under SEM is to be freeze dried and coated with metal vapors like gold, platinum or nickel by using sputter coater. Specimen is then placed in vacuum chamber. When electron beam is focused on the specimen, image forming secondary electrons will be emitted from the metallic surface of the specimen. These electrons will be collected from the detector. This detector generates electron signals. These signals built magnified image of the specimen on a screen of cathode ray tube just like TV camera scan and produce image of the specimen on TV screen.



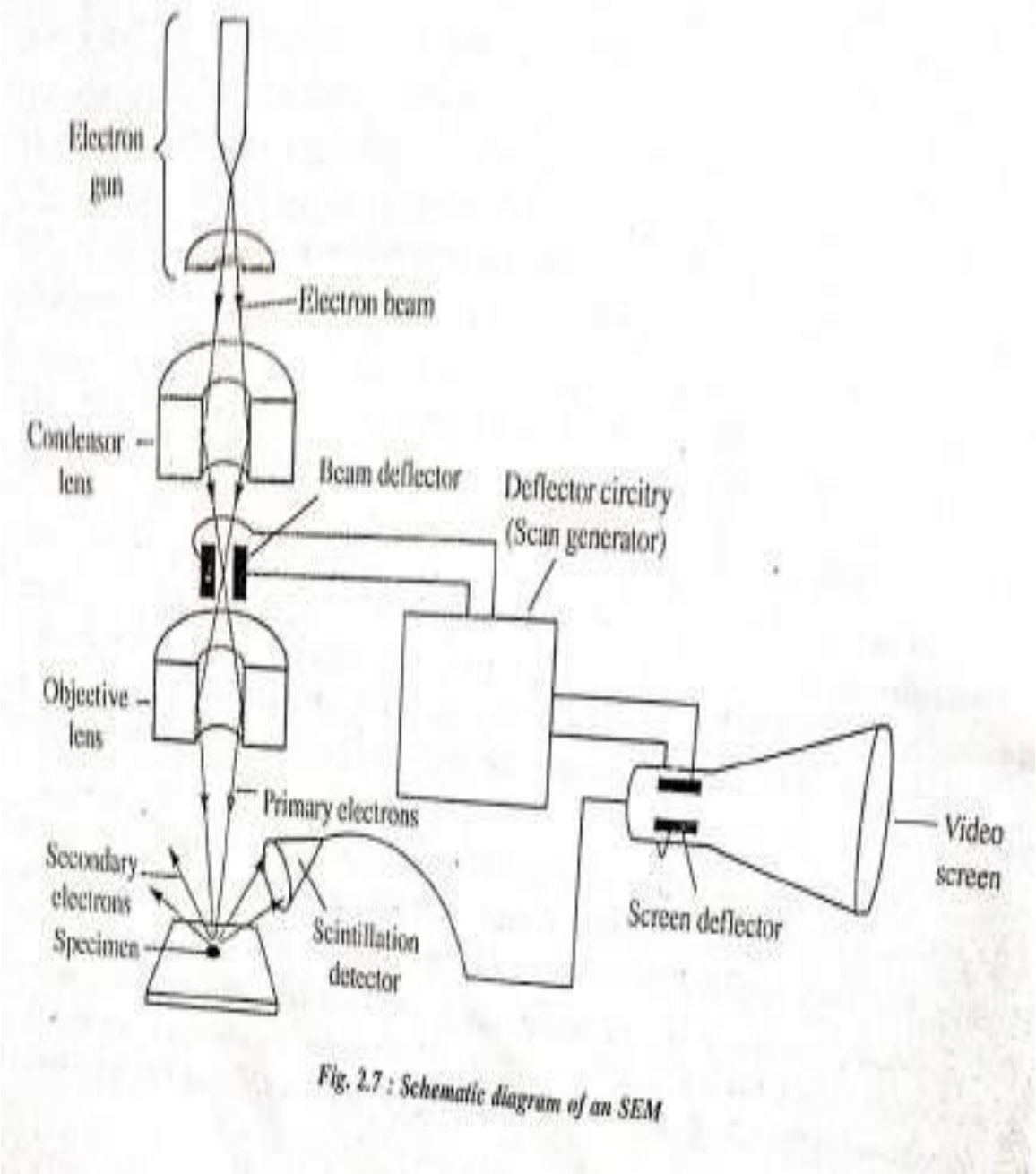


Fig. 2.7 : Schematic diagram of an SEM

STAIN AND STAINING TECHNIQUES

The colouring agents impart colour to the colourless microorganisms. Due to this coloration the microorganisms become visible, so as to observe its cell shape and structure. These stains are composed of a positive and negative ion, one of which is coloured & is known as chromophore. The basic dyes

are in positive ion while acidic dyes in the negative. Since bacteria are towards negatively charged at pH 7.0, thus the coloured positive ion in a basic dye binds to the negatively charged bacterial cell.

Advantages

- The cells are made more clearly visible after they are coloured.
- The differences between cells of different species and within same species can be demonstrated by use of appropriate staining solution.

Chemical substances used to stain microbes are commonly called as 'dyes'. The dyes may be acidic, basic or natural dyes.

The process of staining may involve in in exchange reactions between stain and the active site at the surface of the cell. A large number of dyes are available for staining microbes based on the chemical nature dyes.

Dyes are of two types namely;

- **Acidic dyes**- Eosin, Rose Bengal, Nigros in, Indian ink.
- **Basic dyes**- methylene blue, basic fuchsin, Crystal Violet malachite green.

Fixing: usually fixation is process by which the cell can cell components of microorganisms are preserved in the same position and condition.

Chemical fixation: it is necessary to preserve the cell content and also necessary to preserve the large microorganisms. The chemical fixatives percolate the cell and also the cellular components. They also render the fat and protein inside the cell.

Some of the examples of chemical fixatives are ethyl alcohol, formaldehyde, Mercury chloride, etc.

Heat fixation: in this process, a loop full of bacterial suspension is smeared on the surface of the glass slide in such a way that the cells are separated from one another. Then the slide is gently passed over a flame to dry the smear. Then the bacterial cells are attached to the glass surface.

TYPES OF STAINS

1. *SIMPLE STAINING*

The use of a simple stain for staining organisms is called *simple staining*. The stain like methylene blue, Crystal Violet can be since they have colour bearing ions which are positively charged since most of the bacterial cells are separately charged there is a pronounced attraction between the states and the organism.

Methods of a simple staining

To the glass slide transfer a loopfull of bacterial culture by means of a sterile inoculation loop to the centre of the glass slide, if transfers are made from solid media touch the loop lightly upon the culture and transfer to a drop of water that slightly and mix until a slight turbid results, spread the drop over the slide and allow it to dry. Fix the dried by passing the slide through the flame, slide over 2- 3 times.

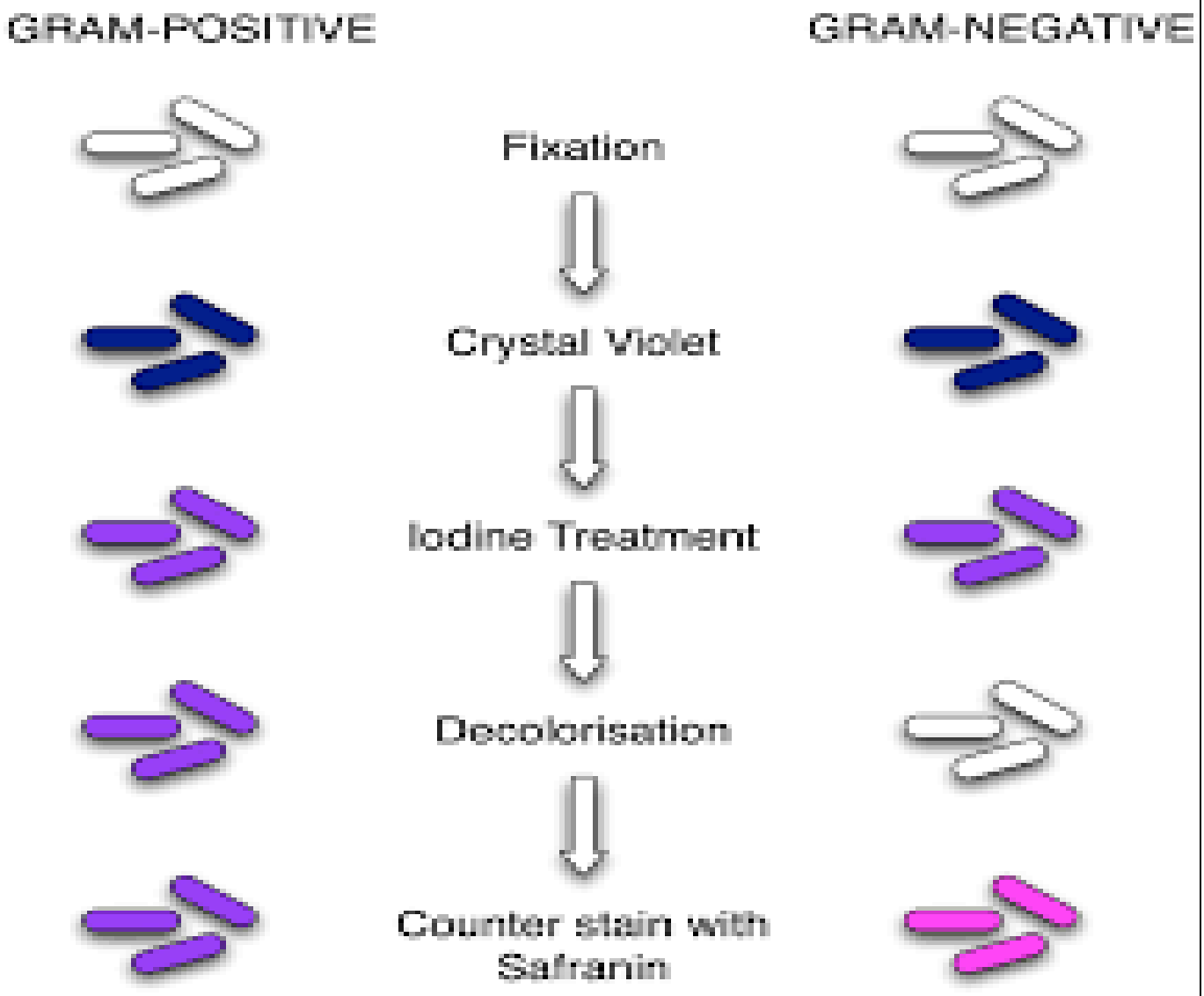
Then flood the fixed smear with several drops of dyes and allow to remain for the following intervals:

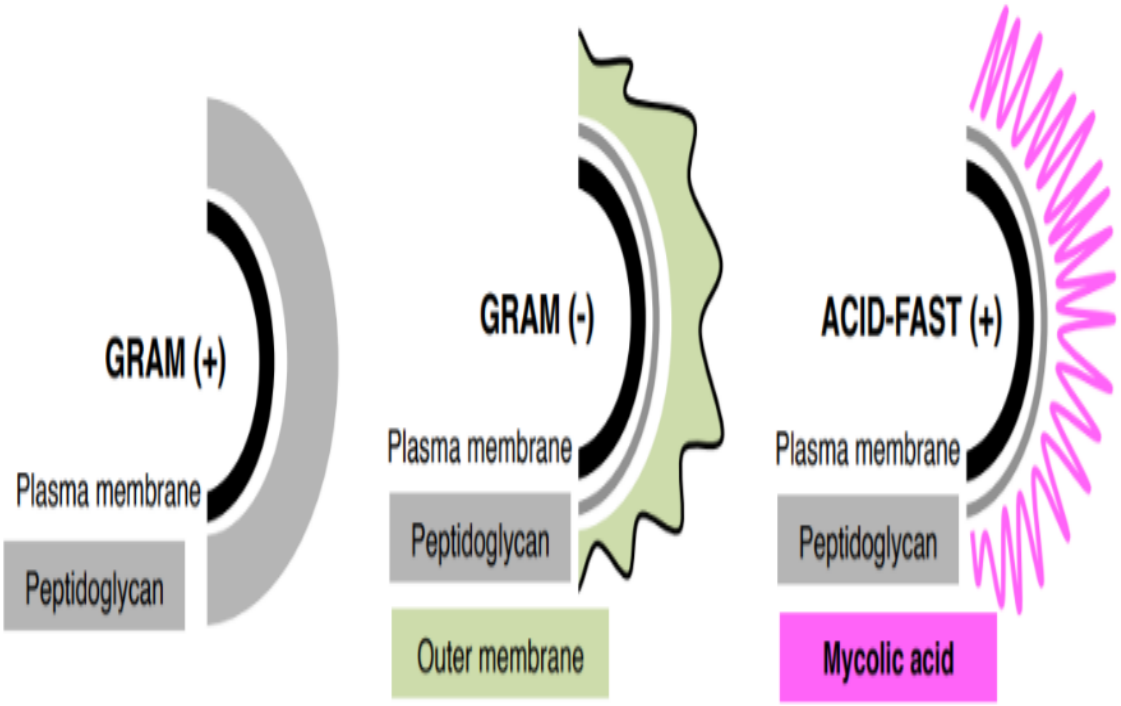
Carbol Fuschin - 10-30 sec Crystal

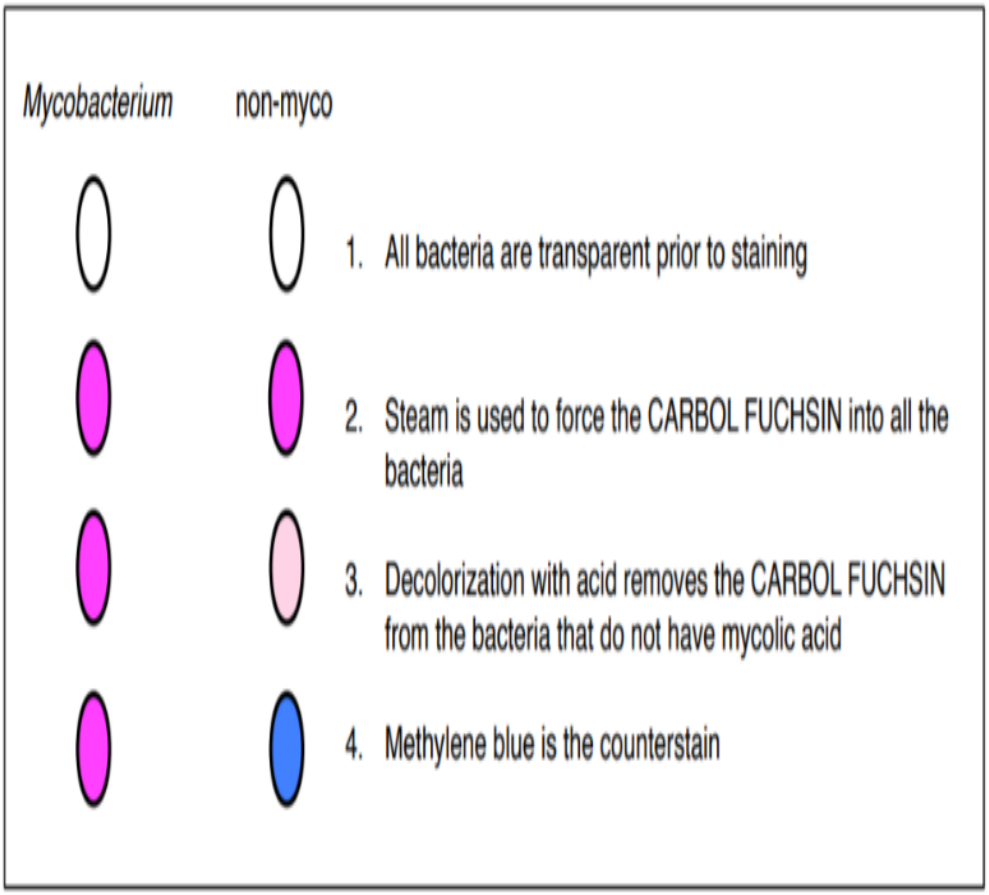
Violet - 30-45 sec

Methylene blue - 3-5 sec

Then wash the slide with excess of water to remove stain and remove the water droplet with the help of blotting paper and then observe under microscope. The simple stain helps us in determining the shape, size and arrangement of the cells in bacteria.




GRAM (+)
Plasma membrane
Peptidoglycan
GRAM (-)
Plasma membrane
Peptidoglycan
Outer membrane
ACID-FAST (+)
Plasma membrane
Peptidoglycan
Mycolic acid


Mycobacterium
non-myco
1. All bacteria are transparent prior to staining
2. Steam is used to force the CARBOL FUCHSIN into all the bacteria
3. Decolorization with acid removes the CARBOL FUCHSIN from the bacteria that do not have mycolic acid
4. Methylene blue is the counterstain

2. *STRUCTURAL STAINING*

Some bacteria are capable of changing in the dormant structure that are metabolically inactive don't grow and early reproduce, since these structures are found inside the cell and thus called the endospore. They are extremely heat resistant and to the radiation. This primary resistant is due to the thick spore coat made up of teichioic acid.

a. *Staining of cell wall*

As we have discuss the simple and differential stains which are mainly cell wall stains and they don't stain any other specific structure such as capsule, flagella, spore etc.

b. *Endospore staining*

Prepare a heat fix smear of the given culture and place the slide on a staining rack above boiling water. Cover the smear with a small piece of paper and saturate with malachite green continue heating for about 5 minutes. Wash gently with water and counter stain with safranin for 30 seconds again wash with water and remove the excess of water with the help of blotting paper. Examine the preparation under oil immersion objective.

This kind of staining is done in case of *clostridium* and *bacillus*.

c. *Capsule staining*

In many bacteria such as *Diplococcus pneumonia, Pseudomonas* etc, a viscous or gelatinous substance secreted by the surrounding of the cell wall called as a capsule.

Method

Prepare smear of the bacterium is made on a glass slide and allow it for air dry (not heat fix) this smear add a drop of Congo red dye and allow it to air dry. Then the smear is fixed with the help of acid alcohol for 15 seconds and washed with water. Then the smear is flooded with fuchsin for one minute then the slide is again washed with water and remove the excess of water with the help of filter paper and examined the preparation under oil immersion objective. This results that the capsule appears colourless surroundi ng red cells against a dark blue background.

Simple staining	Differential staining
1. This method uses only one stain.	This method uses more than one stain.
2. It imparts only one colour to all bacterial cells.	It imparts two or more different colours to bacterial cells.
3. It reveals the size, shape and arrangement of bacterial cells.	It reveals the size, shape and arrangement. In addition, it differentiates two groups of bacteria.
Example: Methylene blue staining method.	Example: 1. Gram's staining method 2. Acid Fast staining method

3. *DIFFERENTIAL STAINING*

In case of this type of staining, the differentiate two types of cells which are belonging to two bacterial species based on their staining characteristics. However, a differential staining can also differentiate the different parts of the cell for instant endospores of flagella etc.

The most popularly known stains in case of differential staining are gram stain and acid -fast stain since this help in differentiating between *gram positive* and *Gram Negative bacteria*.

a. *Gram staining*:

The gram stain is named after Christian gram, a microbiologist who has discovered it in 1884. This staining is particularly used in case of bacteria.

Method of Gram staining

To the heat fix smear add a drop of crystal violet solution, allow it for 1 minute and then wash with tap water, then drink of all the excess of water with the help of a blotting paper and then use iodine solution for 1 minute and again wash with tap water, decolourised with 95% of alcohol and now flood the slide with safranin for 1 minute and again wash with water and air dry, examine the preparation under microscope. usually the gram positive bacteria looks dark purple in colour since they take both stains such as crystal violet and basic fuchsin. While in case of gram negative bacteria look light red being stained only by safranin.

b. Acid fast stain

It was first developed by Paul Ehrlich in year 1882. The acid fast stain, stains only in case of *mycobacteria tuberculosis*, *bacilli* and some related *actinomycetes*. The main stain used in case of this

type is carbolfuchsin which is usually made from basic fashion phenol, water and ethyl alcohol

Method

Method a non-pathogenic species of *mycobacterium* is usually used along with the non-acid fast bacterial, all the cells are stained. The glass slide is heated for about 3-4 minutes. A decolourising solution usually containing the sulphuric acid and ethanol is added. This helps in the removal of extra dye from all the cells except the acid -fast bacteria. However, the methylene blue is added as counter stain.

Summary

1.Optical microscopy

Conventional light microscopy,

Fluorescence microscopy, confocal/multiphoton microscopy

and Stimulated emission depletion microscopy

2. Scanning probe microscopy Scanning tunneling microscopy (STM),

3. Electron microscopy

Scanning electron microscopy (SEM),

Transmission electron microscopy (TEM),

Scanning transmission electron microscopy (STEM),

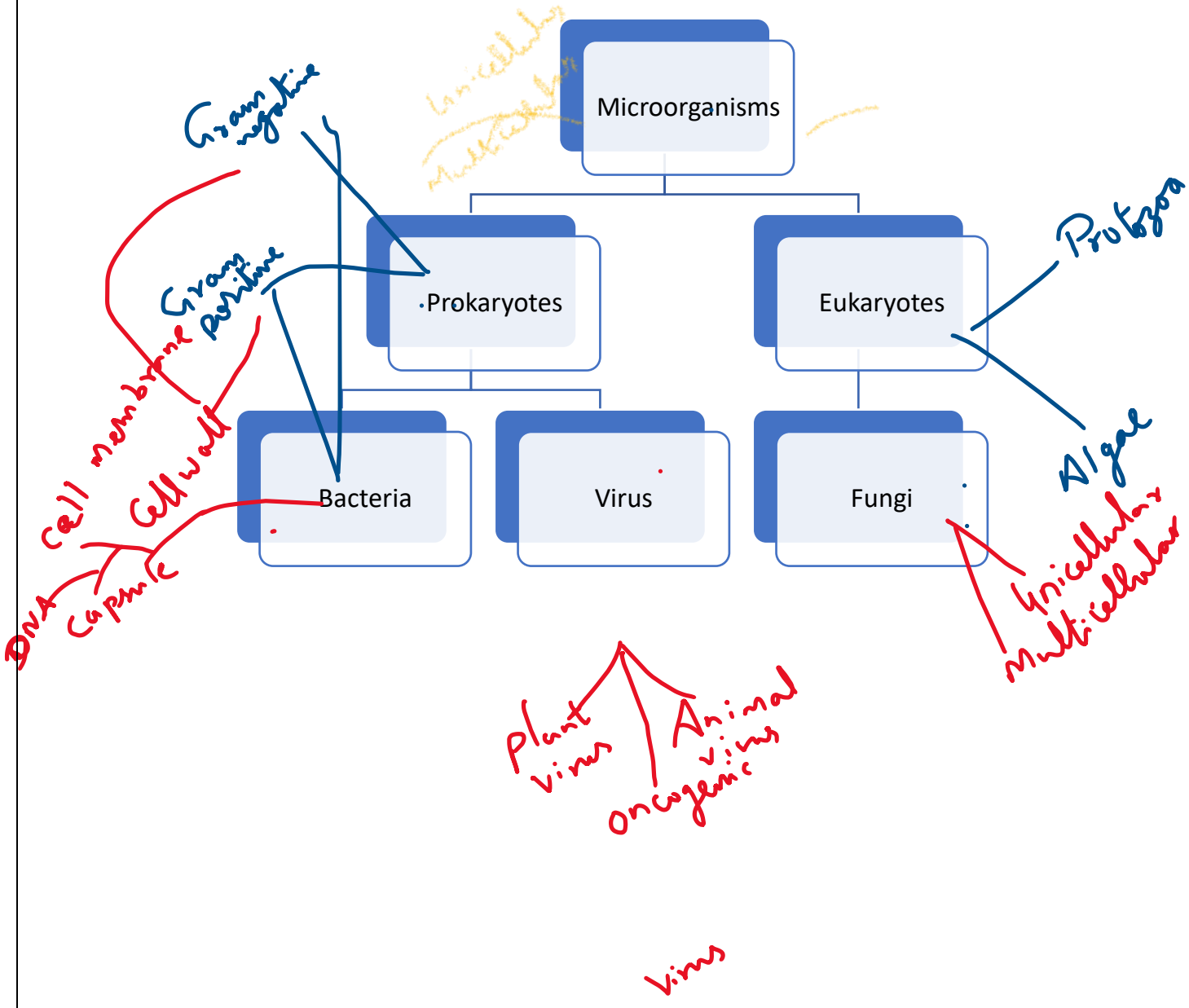
Focus ion beam microscopy (FIB)

The cells are made more clearly visible after they are coloured.

4.The differences between cells of different species and within same species can be demonstrated by use of appropriate staining solution.

5.Acidic dyes- Eosin, Rose Bengal, Nigros in, Indian ink.

6.Basic dyes- methylene blue, basic fuchsin, Crystal Violet malachite green.


Microorganisms
Prokaryotes
Eukaryotes
Bacteria
Virus
Fungi
Gram negative
Gram positive
cell membrane
Cell wall
DNA
Capsule
Protozoa
Algae
Unicellular
Multicellular
Plant virus
Animal virus
oncogenic
virus

UNIT-III

ULTRASTRUCTURE OF A PROKARYOTIC CELL

PROKARYOTES

Prokaryote, also spelled **procaryote**, any organism that lacks a distinct nucleus and other organelles due to the absence of internal membranes. Bacteria are among the best-known prokaryotic organisms. The lack of internal membranes in prokaryotes distinguishes them from eukaryotes. The prokaryotic cell membrane is made up of phospholipids and constitutes the cell's primary osmotic barrier. The cytoplasm contains ribosomes, which carry out protein synthesis, and a double-stranded deoxyribonucleic acid (DNA) chromosome, which is usually circular

Characteristics of Prokaryotic Cell

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

1. They lack a nuclear membrane.
2. Mitochondria, Golgi bodies, chloroplast, and lysosomes are absent.
3. The genetic material is present on a single chromosome.
4. The histone proteins, the important constituents of eukaryotic chromosomes, are lacking in them.
5. The cell wall is made up of carbohydrates and amino acids.
6. The plasma membrane acts as the mitochondrial membrane carrying respiratory enzymes.
7. They divide asexually by binary fission. The sexual mode of reproduction involves recombination.

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.

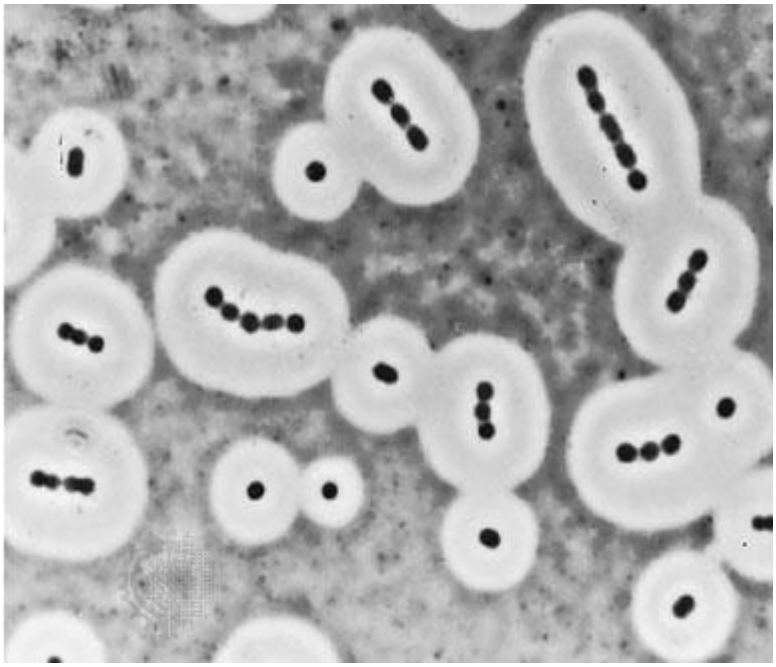
A prokaryotic cell structure is as follows:

1. **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.

Bacterial Capsule:

Capsule

- Capsule is 0.2µm thick viscus layer firmly attached to the cell wall of some capsulated bacteria.
- If capsule is too thick it is known as slime.
- Slime layer are loosely attached to cell wall and can be lost on vigorous washing and on sub culture.
- **Composition of capsule:** 98% water and 2% polysaccharide or glycoprotein/ polypeptide or both.
- In case of Acetic acid bacteria, capsule is composed of homopolysaccharide (hemicellulose)
- *leuconostoc:* capsule is composed of cellulose, consisting of glucose or fructose.
- *Klebsiella pneumoniae:* capsule is made up of glucose, galactose,rhamnose etc.
- In Bacillus anthracis: capsule is made up of Polypeptide (Polymer of D-glutamic acid) and in Streptococci, it is L-aminoacids.
- Capsule is very delicate structure. It can be removed by vigorous washing.
- Capsule is most important virulence factor of bacteria.
- Capsule in visualized by Negative staining technique



There are two types of capsule.

1. **Macro-capsule:** thickness of 0.2µm or more, visible under light microscope
2. **Microcapsule:** thickness less than 0.2µm, visible under Electron microscope

Function of capsule:

1. **Prevent the cell from desiccation and drying:** capsular polysaccharide bind significant amount of water making cell resistant to drying
2. **Protection:** it protect from mechanical injury, temperature, drying etc
3. **Attachment:** capsule helps in attachment on the surface. Eg. *Streptococcus mutants* that cause dental carries attach on teeth surface by its capsule.
4. **Anti-phagocytic** : Capsule resist phagocytosis by WBCs
5. Capsule prevent attachment of bacteriophage on cell surface
6. **Source of nutrition:** capsule is source of nutrition when nutrient supply is low in cell.
7. **Repulsion:** same charge capsulated bacteria repel each other.

Examples of Capsulated bacteria:

Bacillus subtilis

Bacillus anthracis (contains polypeptide capsule)

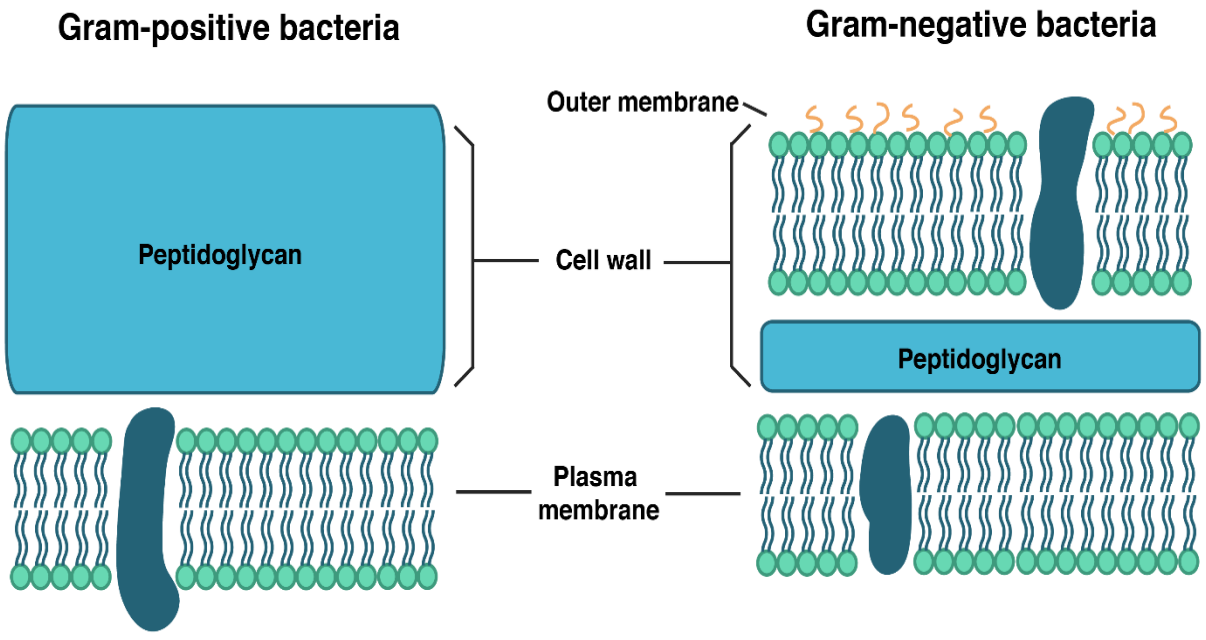
Streptococcus pneumoniae

Klebsiella pneumoniae

Haemplhilus influenza

Cell Wall–

- It is the outermost layer of the cell which gives shape to the cell.
- Cell wall is an important structure of a bacteria.
- It gives shape, rigidity and support to the cell.
- On the basis of cell wall composition, bacteria are classified into two major group ie. Gram Positive and gram negative.



Types of cell wall

1. Gram positive cell wall

Cell wall composition of gram positive bacteria.

1. Peptidoglycan
2. Lipid
3. Teichoic acid

2. Gram negative cell wall

Cell wall composition of gram negative bacteria

1. Peptidoglycan
2. Outer membrane:

- Lipid
- Protein
- Lipopolysaccharide (LPS)

Composition of cell wall:

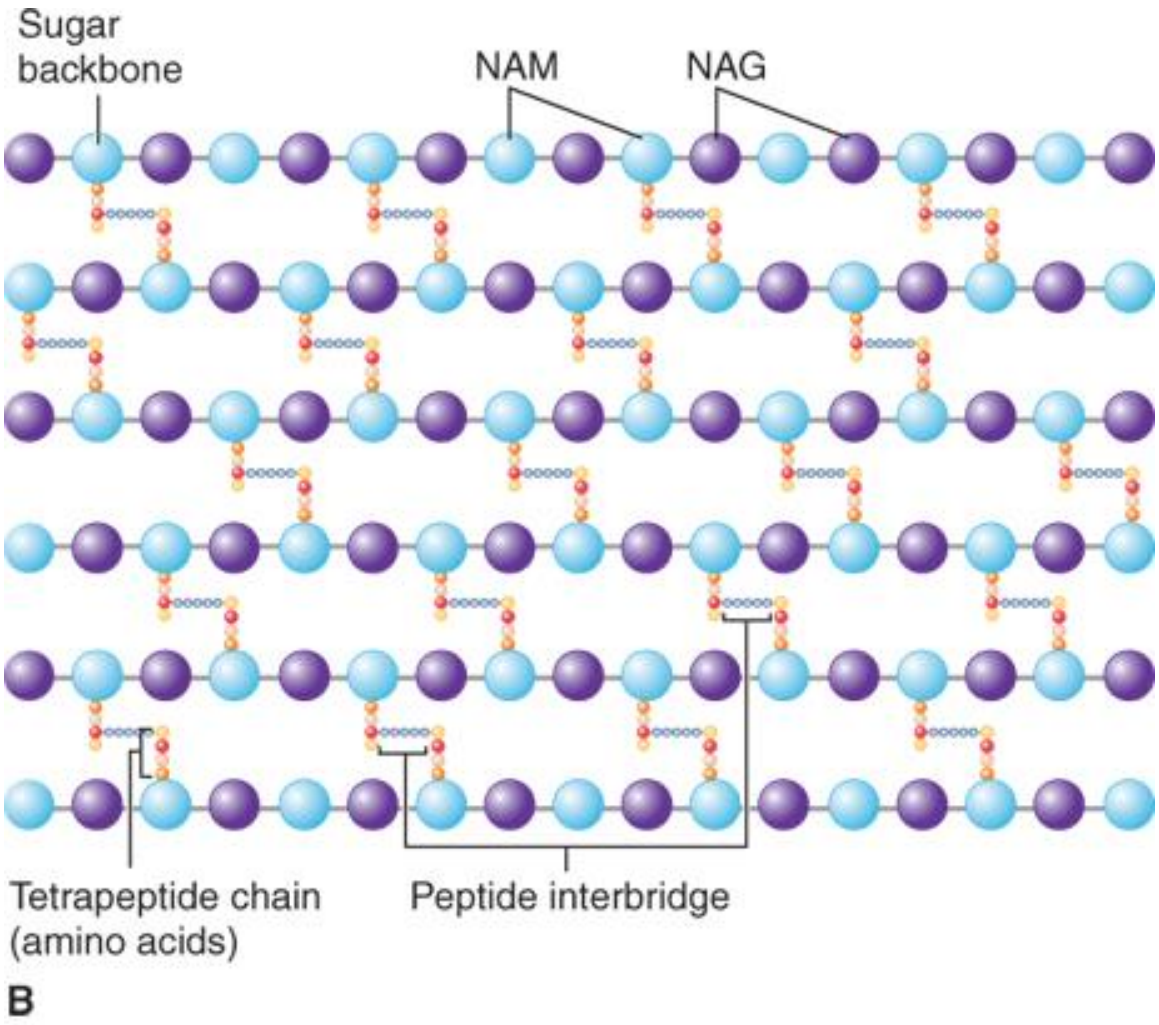
1. Peptidoglycan:

- Peptidoglycan is porous cross linked polymer which is responsible for strength of cell wall.
- Peptidoglycan is composed of three components.

1. **Glycan backbone**
2. **Tetra-peptide** side chain (chain of 4 amino acids) linked to NAM
3. **Peptide cross linkage**

- Glycan backbone is the repeated unit of N-acetyl muramic acid (NAM) and N-acetyl glycosamine (NAG) linked by β-glycosidic bond.
- The glycan backbone are cross linked by tetra-peptide linkage. The tetra-peptide are only found in NAM.

- More than 100 peptidoglycan are known with the diversity focused on the chemistry of peptide cross linkage and interbridge.
- Although the peptidoglycan chemistry vary from organism to organism the glycan backbone ie NAG-NAM is same in all species of bacteria.

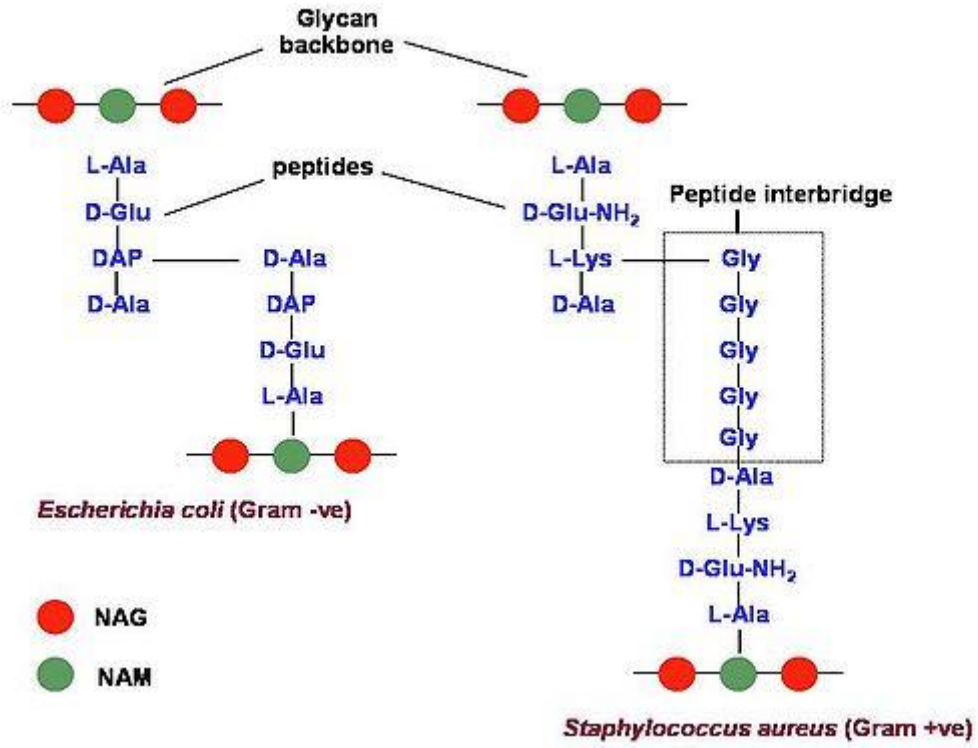


B

The aminoacids found in tetra-peptide are-

- **L-alanine:** 1st position in both gm+ve and gm-ve bacteria
- **D-glutamic acid:** 2nd position
- **D-aminopimelic acid/ L-lysine:** 3rd position (variation occurs)
- **D-alanine:** 4th position

Peptide cross linkage in Gram positive and Gram negative bacteria



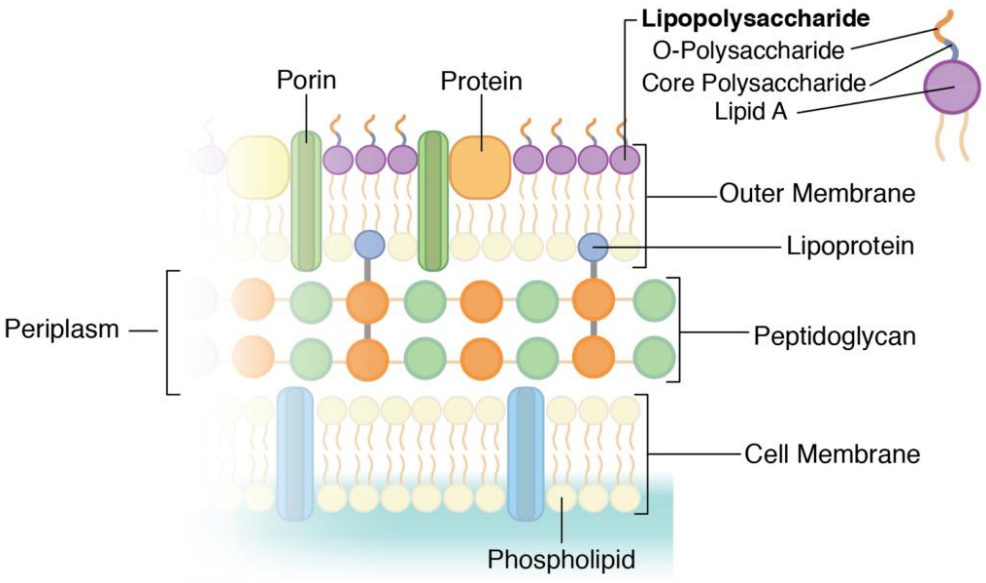
- In gram negative bacteria, peptide cross linkage occur between Diaminopamilic acid (3rd position) of one glycan back bone and D-alanine of adjacent glycan back bone.
- In gram positive bacteria, peptide cross linkage occur by peptide interbridge. The type and number of aminoacids in interbridge vary among bacterial species.

2. Teichoic acid:

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

- It is the major surface antigen of gram positive bacteria.

3. Outer membrane



Gram Negative Bacteria Cell Wall

Figure: Outer membrane in cell wall of gram negative bacteria

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

Function of outer membrane:

- Structure component of gram-ve cell wall
- LPS is an endotoxin produced by gram –ve bacteria
- Lipid-A is antigenic

4. LPS

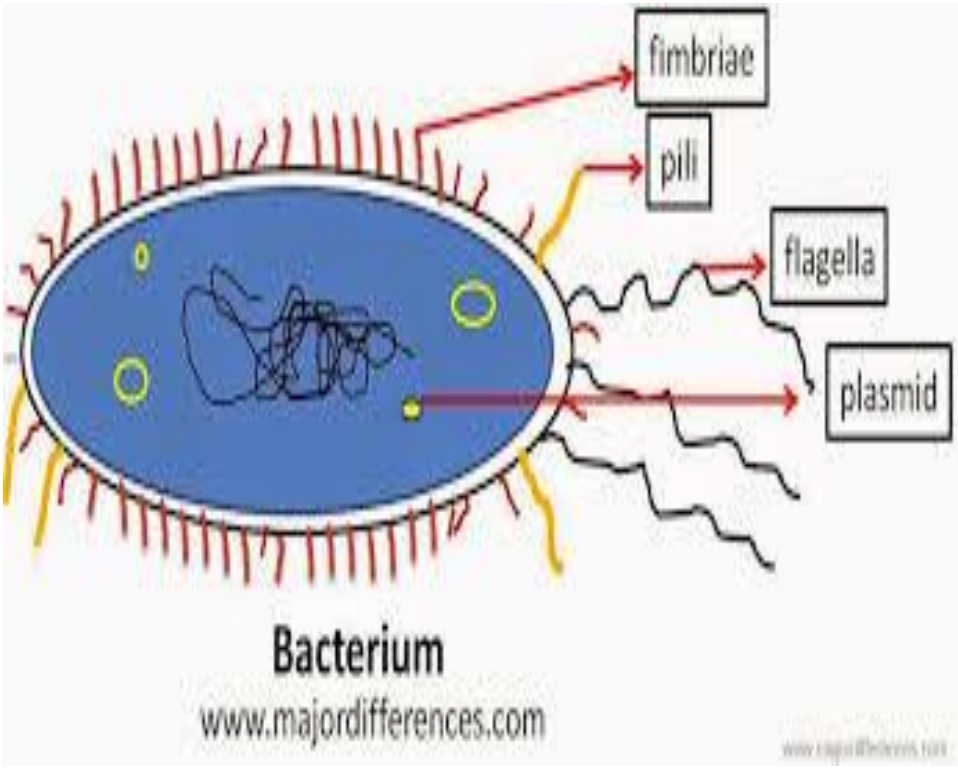
- LPS is attached to outer membrane by hydrophobic bond. LPS is synthesized in cytoplasmic membrane and transported to outer membrane.

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

2.

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

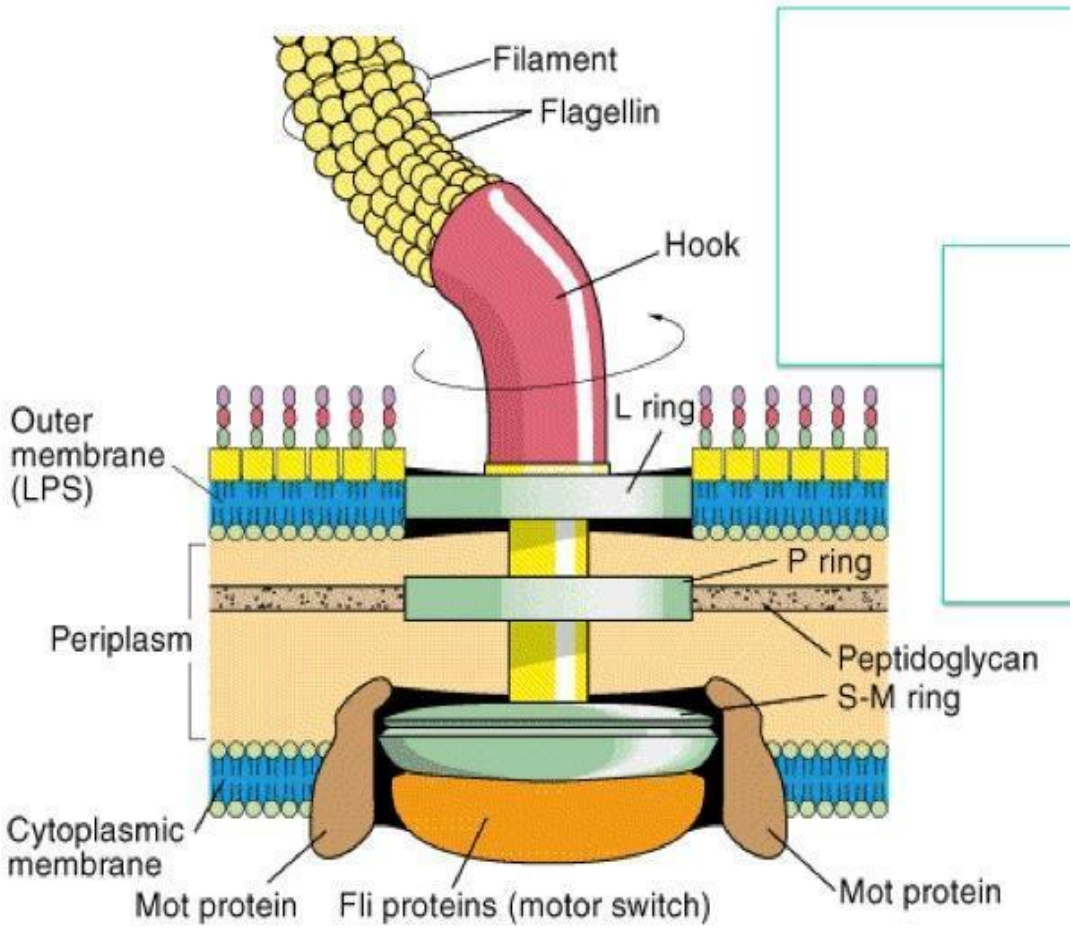
4. **Cell Membrane**– This layer surrounds the cytoplasm and regulates the entry and exit of substances in the cells.



5. **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. **Bacterial Flagella: structure, types and function**
- Flagellum (singular) is hair like helical structure emerges from cell wall and cell membrane
- It is responsible for motility of the bacteria
- Size: thin 15-20nm in diameter.
- Single flagella can be seen with light microscope only after staining with special stain which increase the diameter of flagella.



Structure of flagella:

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

Basal body:

- it is composed of central rod inserted into series of rings which is attached to cytoplasmic memvbrane and cell wall.
- **L-ring:** it is the outer ring present only in Gram -ve bacteria, it anchored in lipopolysaccharide layer
- **P-ring:** it is second ring anchored in peptidoglycan layer of cell wall.
- **M-S ring:** anchored in cytoplasmic membrane
- **C ring:** anchored in cytoplasm

Hook:

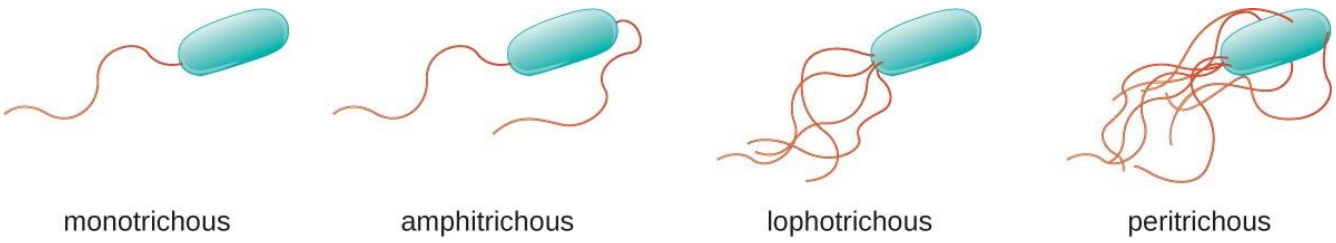
- it is the wider region at the base of filament
- it connects filament to the motor protein in the base
- length of hook is longer in gram +ve bacteria than gram –ve bacteria

Filament:

- it is thin hair like structure arises from hook.

Types of flagella

On the basis of arrangement



1. Monotrichous:

- presence of single flagella in one end of cell.
- examples; *Vibrio cholera, Pseudomonas aerogenosa*

2. Lophotrichous:

- presence of bundle of flagella in one end of cell.
- *example: Pseudomanas fluroscence*

3. Amphitrichous:

- presence of single or cluster of flagella at both end of cell.
- *example; Aquaspirillium*

4. Peritrichous:

- presence of flagella all over the cell surface.
- example; *E.coli, Salmonella, Klebsiella*

5. Atrichous:

- absent of flagella.
- example; *Shigella*

Function:

Flagellar motility:

- At the base surrounding the inner ring (M-S and C ring) there is a series of protein called Mot protein.
- A final set of protein called Fli protein function as motor switch. The flagella motor rotates the filament as a turbine causing movement of the cell in the medium.
- The movement of flagella results from rotation of basal body which is similar to the movement of the shaft of an electric motor.
- A turning motion is generated between S-ring and M ring. S-ring acts as starter while M ring acts as roter.
- The basal body as a whole give a universal joint to the cell and allows complete rotation of hook and filament.
- Flagella moves the cell by rotating the flagella about the basal body. Rotation of flagella is either clockwise or anticlockwise.

6. **Ribosomes**– These are involved in protein synthesis.

7. **Plasmids**– Plasmids are non-chromosomal DNA structures. These are not involved in reproduction. They are self-replicating, extra chromosomal segments of double stranded, circular, naked DNA. Plasmids provide unique phenotypic characters to bacteria. They are independent of main nucleoid. Some of them contain important genes like fertility factor, nif genes, resistance factors and colicinogenic factors. Plasmids can get associated temporarily with nucleoid are known as episomes. Plasmids are used as vectors in genetic engineering.

8. **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.

Inclusion Bodies:

- They are non-living structures present in the cytoplasm.
- The inclusion bodies may occur freely inside the cytoplasm (e.g., cyanophycean granules, volutin or phosphate granules, glycogen granules) or covered by 2-4 nm thick non-lipids, non-unit protein membrane (e.g., gas vacuoles, carboxysomes, sulphur granules, PHB granules).
- On the basis of their nature, the inclusion bodies are of 3 types— gas vacuoles, inorganic inclusions and food reserve.

Inorganic Inclusions:

- Several types of inorganic granules occur in bacteria.
- They include volutin granules, sulphur granules, iron granules, magnetite granules, etc. Because of the ability to pick up different colours with basic dyes, they are called metachromatic granules.
- Two common types of inorganic granules are volutin granules and sulphur granules.
- Volutin granules are polymetaphosphates which function as storage reserve of phosphate. Sulphur granules occur in bacteria living in sulphur rich medium like the one which pick up hydrogen sulphide for obtaining reducing power in photosynthesis.

- Iron granules are similarly found in those bacteria which metabolise iron compounds for obtaining energy.
- *Aquaspirillum magnetotacticum* contains magnetosomes, which are vesicles having magnetite. The granules help the bacteria to orientate themselves along geomagnetic lines

iii)Food Reserve:

- Blue green algae have cyanophycean starch or α-granules, β- granules or lipid globules and cyanophycin or protein granules.
- In bacteria, starch is replaced by glycogen. Neutral fats are absent. Instead poly-beta-hydroxy-butyrate or PBH granules are present.
- A biodegradable plastic can be prepared from PBH. Protein granules are present. Carboxysomes occur in photosynthetic forms.
-

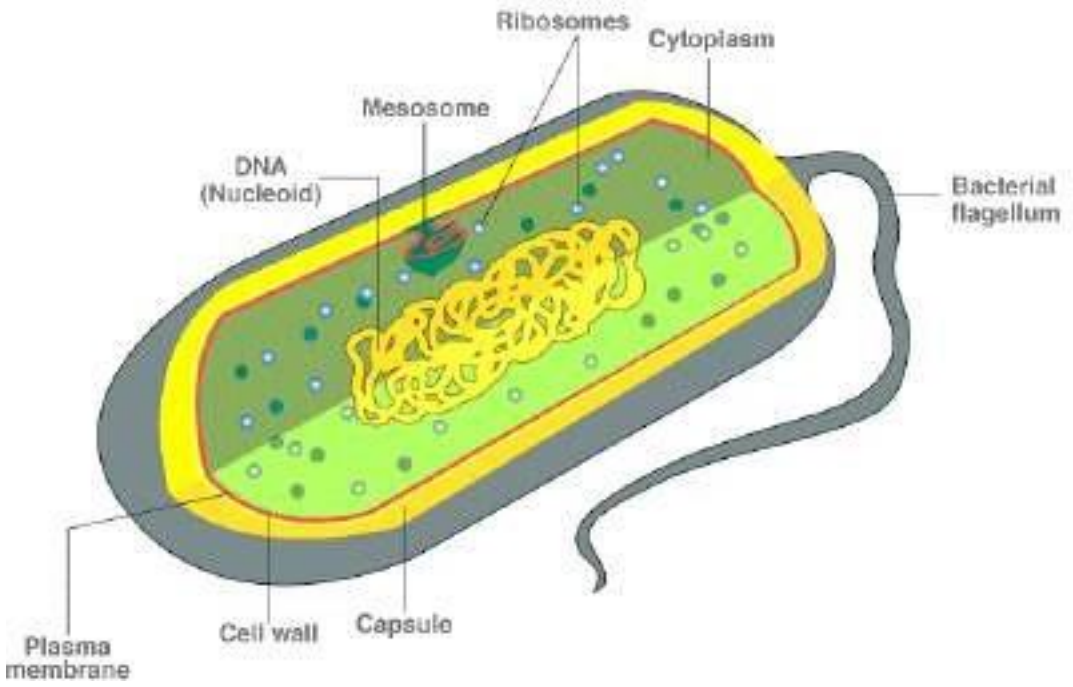
Gas Vacuoles:

They are gas storing vacuoles found in cyanobacteria, purple and green bacteria and a few other planktonic forms. A gas vacuole is without any covering of its own. It consists of a variable number of hexagonal, hollow and cylindrical gas vesicles. Each gas vesicle is surrounded by a single non-unit, non-lipid protein membrane having ribs or folds.

The membrane is impermeable to water but is permeable to atmospheric gases. Gas vacuoles protect the bacteria from harmful radiations. They also constitute buoyancy regulation mechanism for their proper positioning in water during daytime for photosynthesis.

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.



Prokaryotic Cell Diagram illustrates the absence of a true nucleus

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.

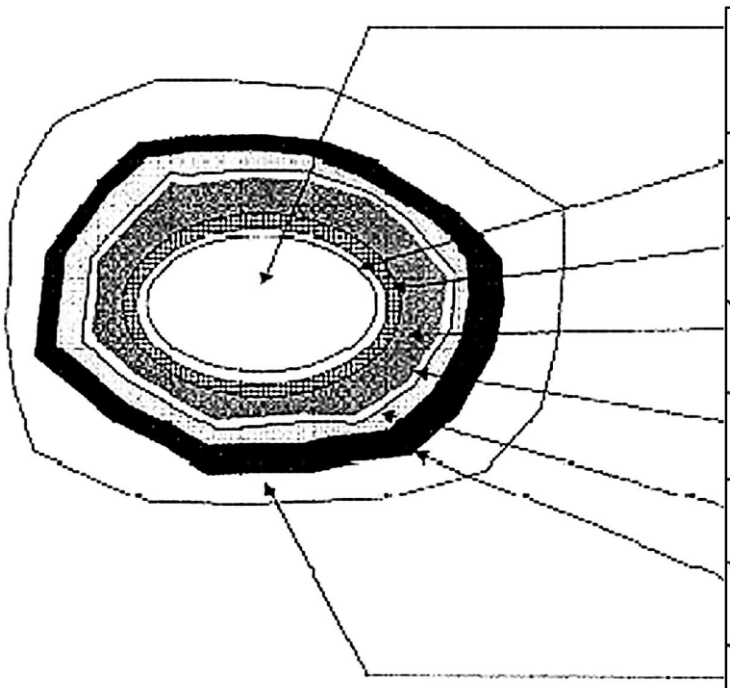
Bacterial Spore: structure, types,

- Spore is metabolically dormant structure produced during unfavorable condition by the process called sporulation

- Sporulation occur during late log phase or early stationary phase
- Under favorable condition spores germinate to give vegetative cell.

- Size: 0.2 µm
- Spore are resistant to nutrition starvation, temperature, extreme pH, antibiotics etc

Structure of endospore:



Core	DNA, RNA, Proteins, SASPs, DPA, Ca^{2+}
Inner membrane	Lipid/Protein
Germ cell wall	Peptidoglycan
Cortex	Modified peptidoglycan
Outer membrane	Lipid/Protein
Inner spore coats	Proteins
Outer spore coats	Proteins
Exosporium	Proteins

An endospore has following layers

1. Exosporium
2. Spore coat
3. Cortex
4. core

Exosporium:

- It is the outermost layer made up of protein that encloses spore coat.
- In some bacterial spore, exosporium is made up of polysaccharide and lipid.

Spore coat:

- It is thick double layered covering that encloses cortex

- Spore coat consists of spore specific protein, mainly contains cysteine and hydrophobic amino acids. Due to presence of these aminoacids,spore are resistant to adverse environmental condition.

Cortex:

- Inside the Spore coat, there is cortex made up of loosely arranged peptidoglycan layer.
- Inner layer: comprises about 20% of peptidoglycan, it is tightly arranged
- Outer layer: it is loosely arranged, it can be hydrolysed during spore germination.it comprises alalnine (55%), tetra-peptide (15%) and muramic lactum (30%).

Core:

- It is the innermost part of spore
- It is also known as spore protoplast
- Core consists of core wall, cytoplasmic membrane, cytoplasm, nucleoid, ribosomes and other cellular materials.
- Core contains (10-25%) water so, the cytoplasm is gel like
- It contains high amount of calcium and dipicolinic acid in the form of calcium dipicolinate (10-15% by dry weight).
- Core also contains high percentage of small acid soluble protein (SASP).
- SASP is synthesized during sporulation and it binds to DNA in core and protect it from potential damage caused by UV radiation, desiccation and drying.
- In addition, SASPs also provides nutrition and energy for spore germination.

Types of bacterial spore

1. Endospore:

- It is produced within the bacterial cell.
- Bacteria producing endospore are: *Bacillus, Clostridium, Sporosarcina* etc

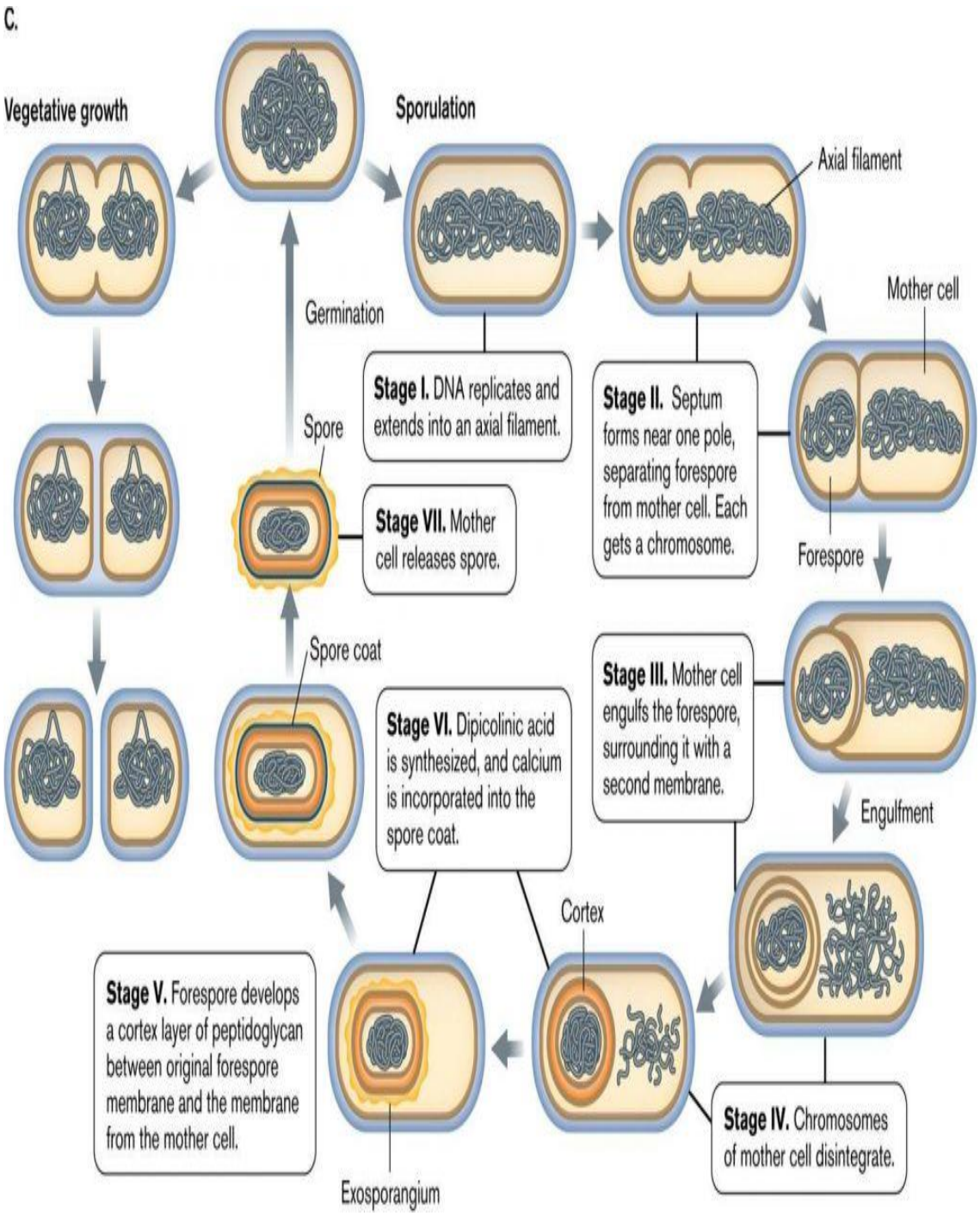
2. Exospore:

- It is produced outside the cell
- Bacteria producing exospore: *Methylosinus*

Sporulation

- During unfavorable condition, vegetative cell converts into spore by the process known as **sporulation**
- Sporulation can be divided into several stages. In *Bacillus subtilis*, entire process of sporulation takes 8 hours to complete from stage 0 to stage VII

Stages of sporulation:



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 bacterial cell divide equally into two, following replication of DNA. The cell wall and cytoplasm also split resulting in the formation of two daughter cells.

Sexual Reproduction: In bacterial sexual reproduction there is no meiosis, formation of gametes and zygote. Instead, it involves transfer of a portion of genetic material (DNA) from a donor cell to a recipient cell. This process is called as genetic recombination or parasexuality. It is known to occur in the following three ways:

Transformation: In this process, one kind of bacterium is transformed into another kind. It takes place by a transfer of DNA from a capsulated bacterium into a non-capsulated bacterium. It has been observed in Diplococcus bacteria.

Transduction: In this process, DNA of a bacterial cell (donor) is transferred into another bacterial cell with the help of a bacteriophage. This process is known to occur in several bacterial species such as Salmonella, Escherichia, Micrococcus and Stigella.

Conjugation: It is a process in which the genetic material of a bacterial cell of a particular strain is transferred into that of another bacterial cell of a different strain. Of the two strains of bacteria involved, one acts as donor (or male) and the other as a recipient (or female). The donor cells are known to possess a sex factor or fertility

factor (F factor) as a component of its circular DNA (F+ strain). The recipient cell does not have this factor and hence it is described as F- strain. A conjugation between cells of F+ and F- strains always results in the formation of F+ bacterial cells in the progeny.

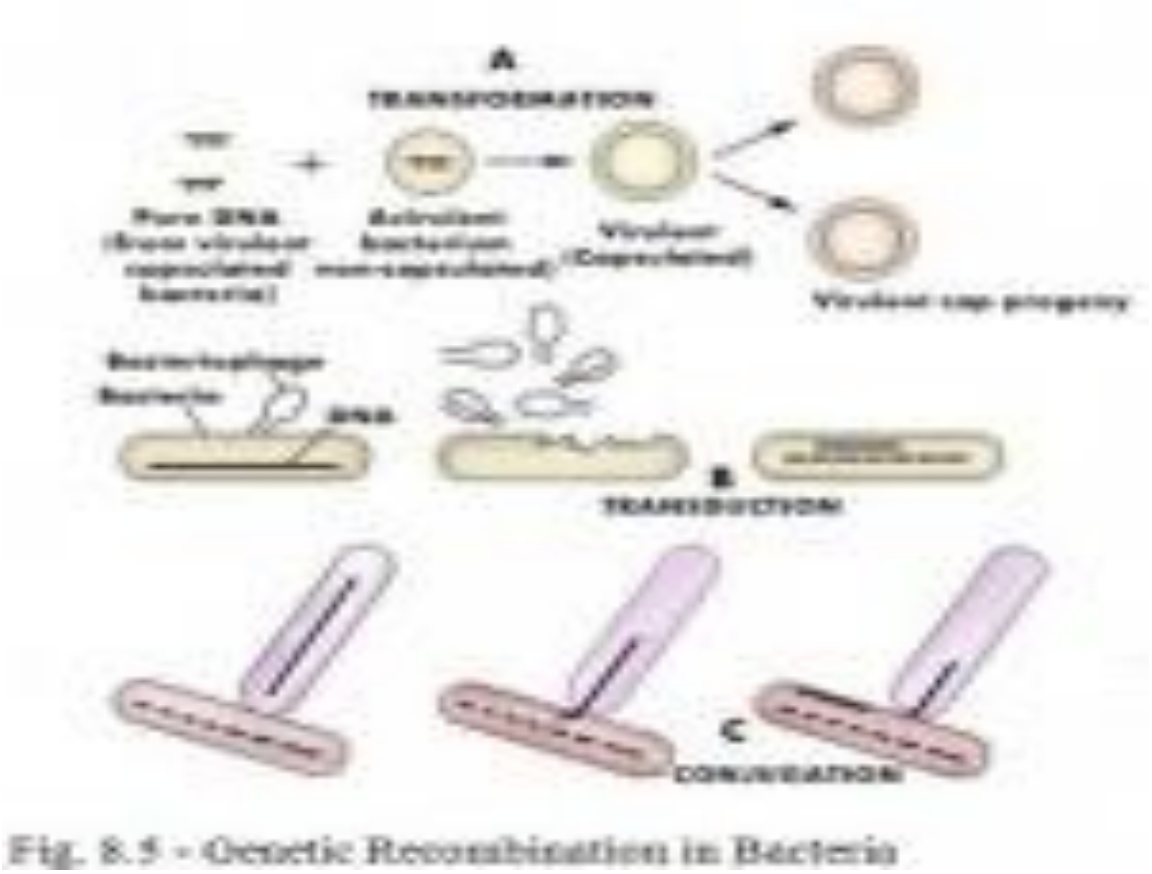


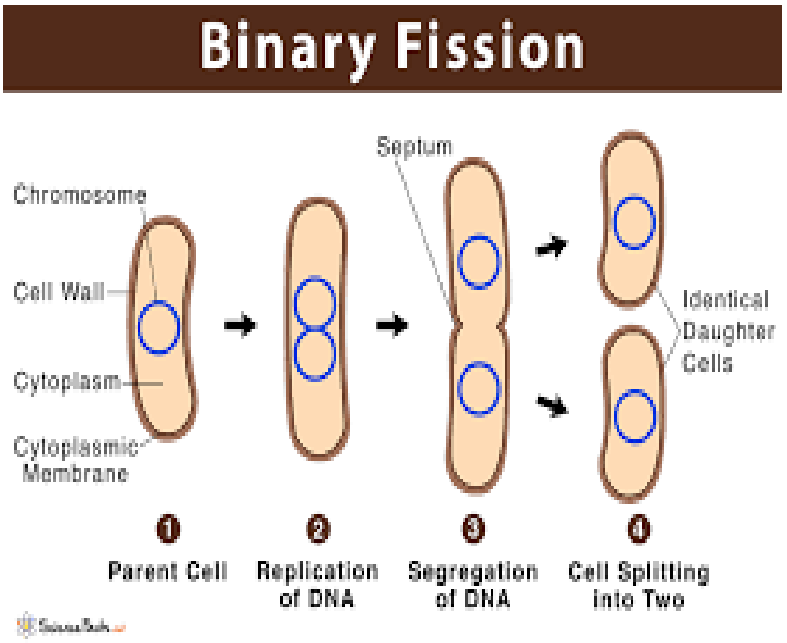
Fig. 8.5 - Genetic Recombination in Bacteria

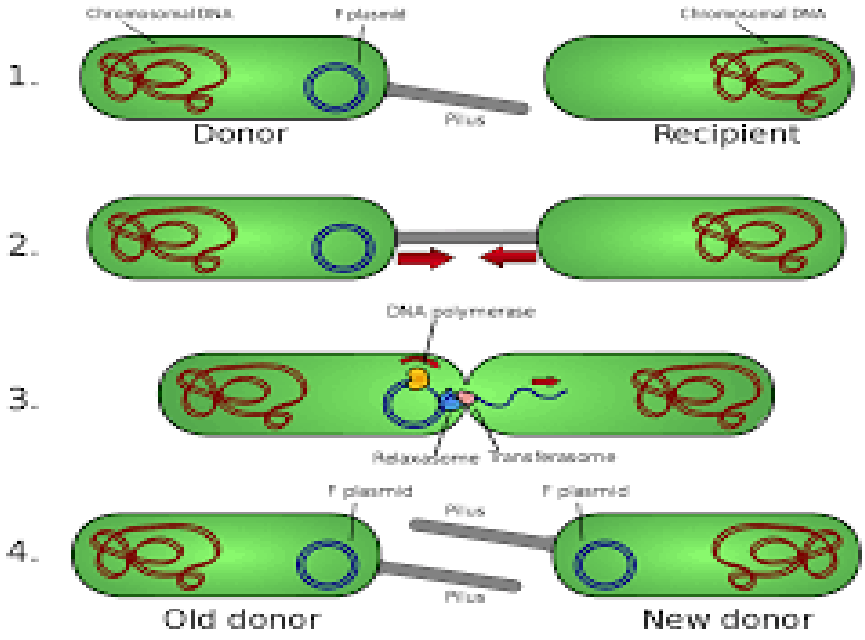
2. Asexual reproduction

A) Binary fission: It is the most common mode of asexual reproduction. The cytoplasm and nucleotide of a bacterial cell divide equally into two, following replication of DNA. The cell wall and cytoplasm also split resulting in the formation of two daughter cells.

Unlike in multicellular organisms, increases in cell size and reproduction by cell division are tightly linked in unicellular organisms. Bacteria grow to a fixed size and then reproduce through binary fission, a form of asexual reproduction. Under optimal conditions, bacteria can grow and divide extremely rapidly, and bacterial populations can double as quickly as every 9.8 minutes. In cell division, two identical clone daughter cells are

produced. Some bacteria, while still reproducing asexually, form more complex reproductive structures that help disperse the newly formed daughter cells Binary fission in bacteria DNA





VIRUSES

The word virus (Latin word= a poisonous liquid) was used to denote all kinds of poisonous agents including snake poisons and till about 140 years ago

The inhabitants of microorganisms have lot of influence on human life. They are both friends as well as enemies of man. Among the most important of microbial enemies of human living beings, viruses occupy a prominent place. Simple and tiny to pass through the minute bacterial filters, yet potent enough to change the destiny of human life, viruses are today the centre of attention in causing one of the deadliest human diseases.

Adolf Meyer demonstrated the existence of virus. They are a cellular as they lack cellular organisation.

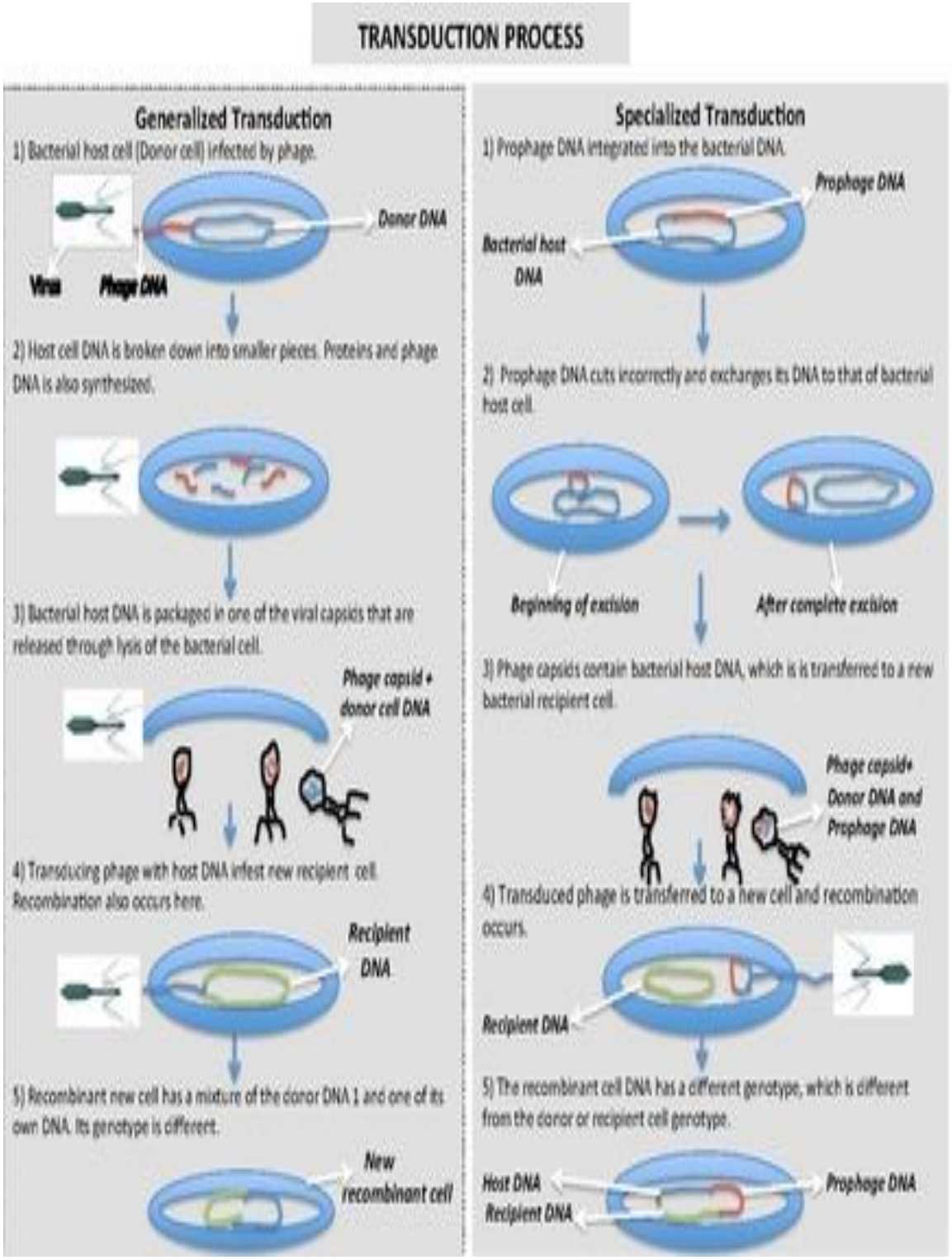
Living characters of viruses

- Viruses have genetic material DNA are
- They mutate.
- They can grow.
- They can be transmitted from one host to another.
- Capable of multiplication within a host.
- They react to heat, radiation and Chemicals.
- They show irritability.
- They bring about enzymatic changes *in vitro*.
- They are able to infect and cause diseases to living beings.
- The DNA and proteins of viruses are similar in composition and structure those of higher organisms.

Non-living characteristics of viruses.

- They can be crystallized like an ordinary chemical and stored in a bottle are test tube.
- Outside the host, viruses are inert (inactive).
- There is no cell wall, membrane or cytoplasm.

- Sedimentation of viruses is according to their molecular weight like non-living beings.



- They are not capable of any function, unless they obtained metabolic products from others.
- Energy production enzyme system is absent.

Some unique characteristics of viruses (living and nonliving):

- Presence of only DNA RNA.
- Capacity to produce from the nucleic acid.
- Do not show cell division.
- They use the metabolic machinery of the host cell to replicate.

Hence many biologists believe it is better to regard 'viruses as chemicals in a test tube but living are in inside the host'.

Properties of viruses:

- Viruses are called acellular as they do not have cellular organisation like other microorganisms.
- They are ultramicroscopic (invisible under ordinary microscope).
- Genetic material is either DNA or RNA but never both.
- They are obligate parasites.
- Viruses can pass through bacterial filters.
- Even though viruses are made up of nuclear proteins, they lack the enzymes necessary for the synthesis. For this they depend on host enzyme.
- Viruses do not show cell division.
- They can be crystallized.

- Virus proteins have high molecular weight.
- They can be transmitted from one host to another either directly or indirectly through vectors.
- The capsid (outer coat) of viruses is mostly made up of proteins except in some animal viruses where polysaccharides are also present.
- They can resist high temperature and they are also resistant to acids, alkalis and salts.
- They are capable of mutation.

Classification of viruses

Earlier viruses were classified based on their hosts:

- Animal virus- these insect animals.
- Plant viruses- these infect plants.
- Bacteriophages- these infect bacteria
- Phyco viruses- these infect algae.
- Mycoviruses- these insect fungi
- Mycoplasm viruses- these infect mycoplasma
- Cyanophages- infect cyanobacteria

Lowaff in 1966 proposed systematic classification according to which viruses are classified based on the following criteria:

1. Chemical nature of nucleic acid: RNA or DNA- single or double stranded.

2. Structure of capsule: helical, icosahedral, naked or enveloped.
3. Site of replication: nucleus or cytoplasm.
4. Host range: specific host tissue or cell types.
5. Mode of transmission: body fluids, feces, water etc.
6. Specific surface structures: antigenic properties.

Lowaff, Home and Toumier proposed the following syatem of classification; according to which the viruses are included under the Phyla- Vira which is divided in to 2 sub phyla:

- *Subphylum*- Deoxy-vira (DNA virus)
- *Subphylum*- ribovira (RNA virus).

In other system of classification proposed by Layiens and King in 1975, viruses are classified based on the type of nucleic acid, presence or absence of an envelope and site of assembly of virus particles in the host.

They have classified viruses into 4 divisions:

- ssRNA viruses (single stranded RNA viruses)
- dsRNA viruses (double stranded RNA viruses)
- ssDNA viruses (single stranded DNA viruses)
- dsDNA viruses (double stranded DNA viruses)

STRUCTURE OF VIRUSES

Viruses are acellular, hence they don't have cytoplasm, Cell wall and cell membrane. They are extremely small, smaller than the bacteria.

The technical name of virus particle is **Virion**. Virion varies in size. They range in size from small 20 nm of parvo virus to the 250 nm of Pox viruses may appear in several shapes. They may have rod shaped, Bullet shaped, oval and pleomorphic.

Helix: certain viruses are helix and are said to have helical symmetry. Their helix is a tightly wound coil resembling a cork screw or spring.

Icosahedron: it is a polyhedron with triangular faces and 12 corners.

Complex: these viruses have a combination of both helical and Icosahedral symmetry.

All the Viruses consist of two basic components, a core of nucleic acid called the *genome* and a surrounding coat of proteins known as a capsid. The genome contains either DNA or RNA, but not both the nucleic acids may be single stranded or double stranded which may be broken or unbroken.

The capsid protects the genome. It also gives regular symmetry to the virus. The capsid is subdivided into individual protein subunits called *capsomeres*. The capsid and genome is called *nucleo-capsid.*

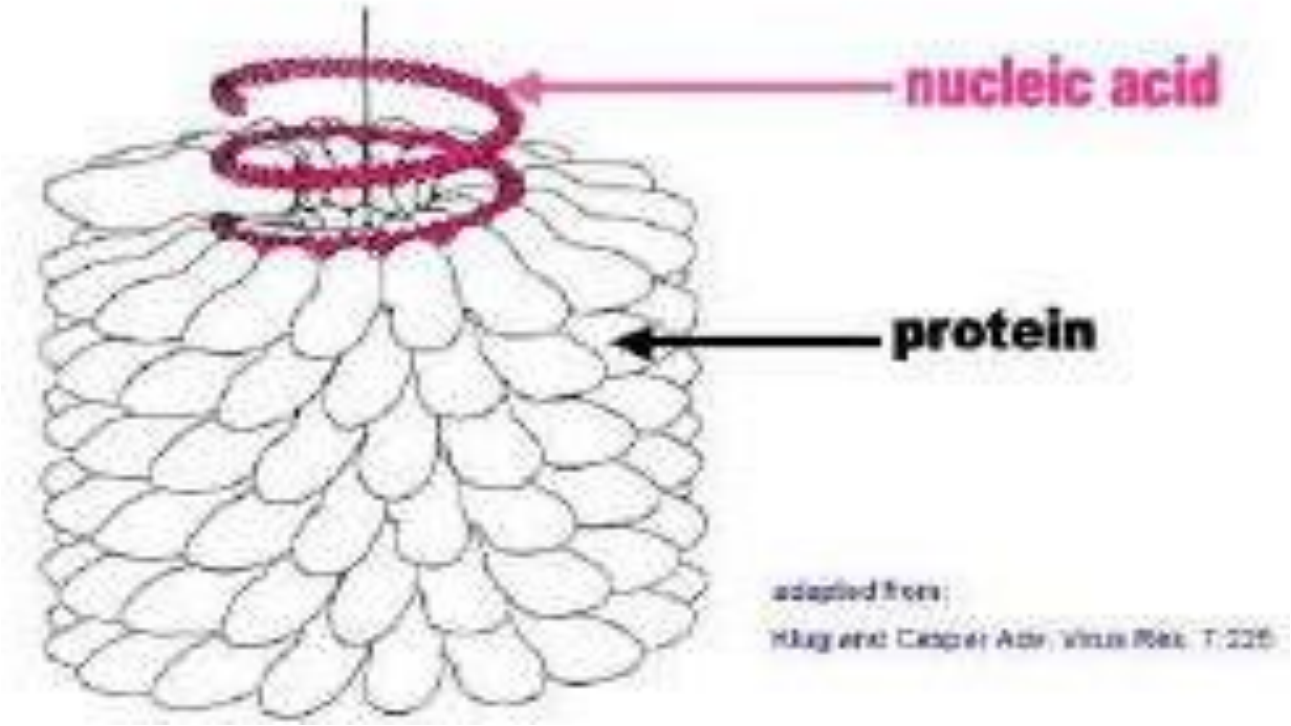
❖ PLANT VIRUSES

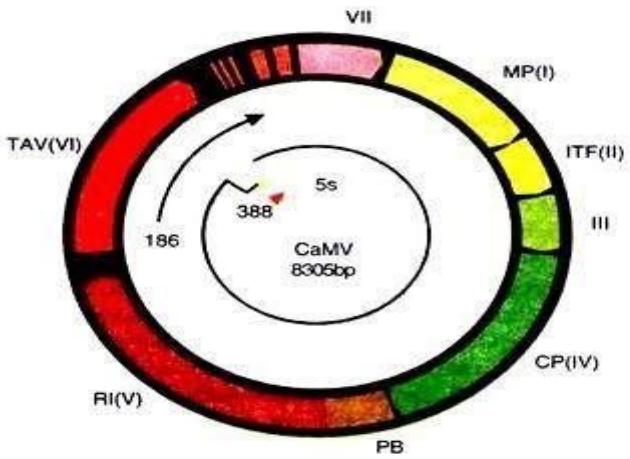
Plant viruses are the viruses that infect Plants. Most of the plant viruses are RNA viruses, while only few plants contain the DNA for example Cauliflower Mosaic Virus (CaMV). In case of this, the structure of the DNA is double helical structure while the structure of the RNA may be either single stranded or

double stranded RNA.

The earliest viruses to be identified and described are viruses infecting plants and one of the most thoroughly studied is Tobacco Mosaic Virus (TMV). Generally the plant viruses are either elongated or spherical in shape.

TOBACCO MOSAIC VIRUS





Open circular, double stranded DNA showing seven ORFs. The genome contains discontinuities in both strands, one in the transcribed strand and 1 to 3 in the non-transcribed strand

TMV- TOBACCO MOSAIC VIRUS

- The TMV is one of the most thoroughly studied viruses in case of plants.
- The TMV is a rod shaped helical virus.
- It is 300 Å long and around 15 - 17 nm in diameter.
- Structurally there is a single stranded RNA (ssRNA) helix with a helical protein sheath. T
- The virus weight is about 38 X 10^6 Dalton's.
- According to the X-ray crystallographic study it shows that the protein sheath is made up of about 2,000 identical subunits and each subunit has a molecular weight of about 12 -15000. Generally the shapes of the subunits are ellipsoidal.
- The genetic material which is present in the centre which is made up of a single stranded RNA molecule with a molecule weight of about 2.1 million. The pitch of The Helix is about 23 Å while the radius is 39 Å.

CaMV- CAULIFLOWER MOSAIC VIRUS

- The cauliflower mosaic virus was the first plant virus to show the presence of the DNA molecule instead of RNA.
- CaMV infects Cauliflower plants.
- CaMV is an icosahedral virus about 24 nm in diameter.
- It has about 10 to 12% double stranded DNA and rest of the proteins and amino acids.

- Its protein shell is made up of 180 protein subunits and clustered into groups of 5 (pentameres) and 6 (hexameres).
- The diameter is 25-30 nm.
- Weight is 5.6 X 10^8 daltons.

❖ ANIMAL VIRUSES

the viruses that infect animals are called animal virus, once they are infected to animal they shows a variety in their structure and the host range however there is a considerable range size of viruses. But most of the viruses fall in the size range between 10 and 200 nm. while some like yellow fever foot and mouth disease are small in diameter of about 25 nm where size in others like Pox virus are large about 230 nm in diameter, however except these large viruses, while most of the others are invisible.

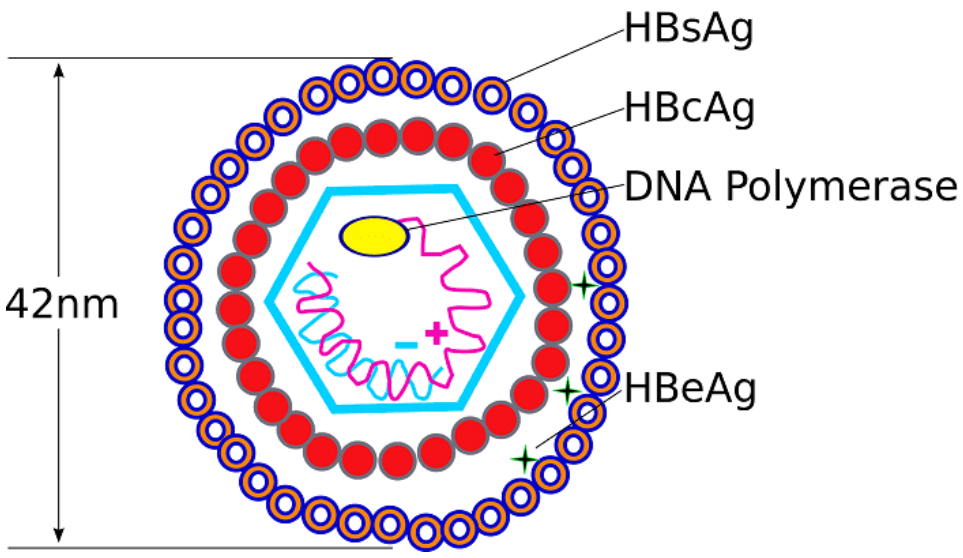
In other outward form i.e., shape of viruses will also differ from one virus to other. Generally they may be rod shape, spherical to oval or Bullet shaped.

In general majority of the animal virus possesses a membranous envelope outer to the protein coat which encloses the genetic material (nucleic acids). The virus membrane mainly consists of the fats and glycoproteins and which play an important role in recognising in host cell surface during infection and the genetic material made of either RNA or DNA.

Some of the examples of DNA viruses are small pox viruses, Pox virus has come up herpes virus and hepatitis B virus.

HEPATITIS- B VIRUS

- The Heptitis-B causes a disease called as the serum hepatitis.
- Can be transmitted by sexual contact or even from the mother to the Infant during the child birth.
- The disease can also be caused by saliva which is the other means of transfer of the virus.
- The period of Heptitis-B is around 100-200 days.
- The virus is more dangerous than the HIV virus and this Heptitis-B also leads to death.
- Heptitis-B belongs to the family Hepadnaviridae.
- Genetic material is DNA.
- The genetic engineering process has also used for the synthesis of vaccine for Heptitis-B.
- The vaccine is marked by different companies as recombivax.
- Preservation is through vaccination.



HIV- HUMAN IMMUNODEFICIENCY VIRUS

- HIV belongs to the lentivirus which is subgroup of retroviridae.
- The virus has a spherical envelope and it is about 90-115 nm in size.
- This causes acquired immunodeficiency syndrome (AIDS), a condition in humans in which progressive failure of immune system.
- Classification:
  - Family: Retroviridae
  - Subfamily: Orthoretrovirinae
  - Genus: lentivirus
  - Species: HIV 1/ HIV 2
- Very high genetic variability.
- Composed of two copies of positive single stranded RNA conical capsid composed of viral protein P24.
- The RNA genome consists of 9 genes.
- Inside of capsid are three enzymes required for HIV replication: reverse transcriptase, integrase and protease.
- Matrix is surrounded by phospholipids.
- HIV doesn't survive well outside the body.
- May survive upto 7 days in dry blood.
- Virus is inactivated under extreme changes of pH in acidic and alkaline medium.

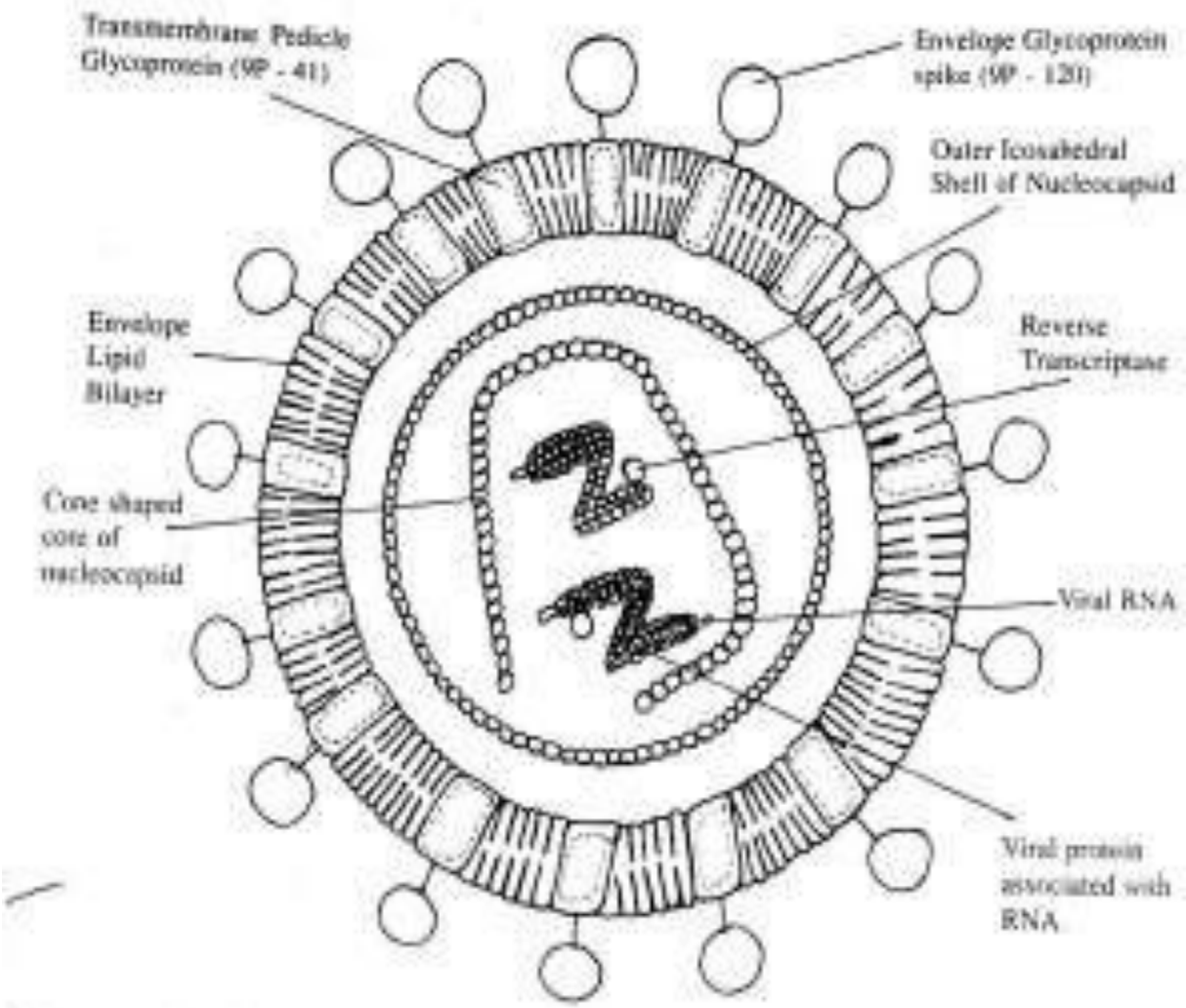
❖ BACTERIOPHAGES

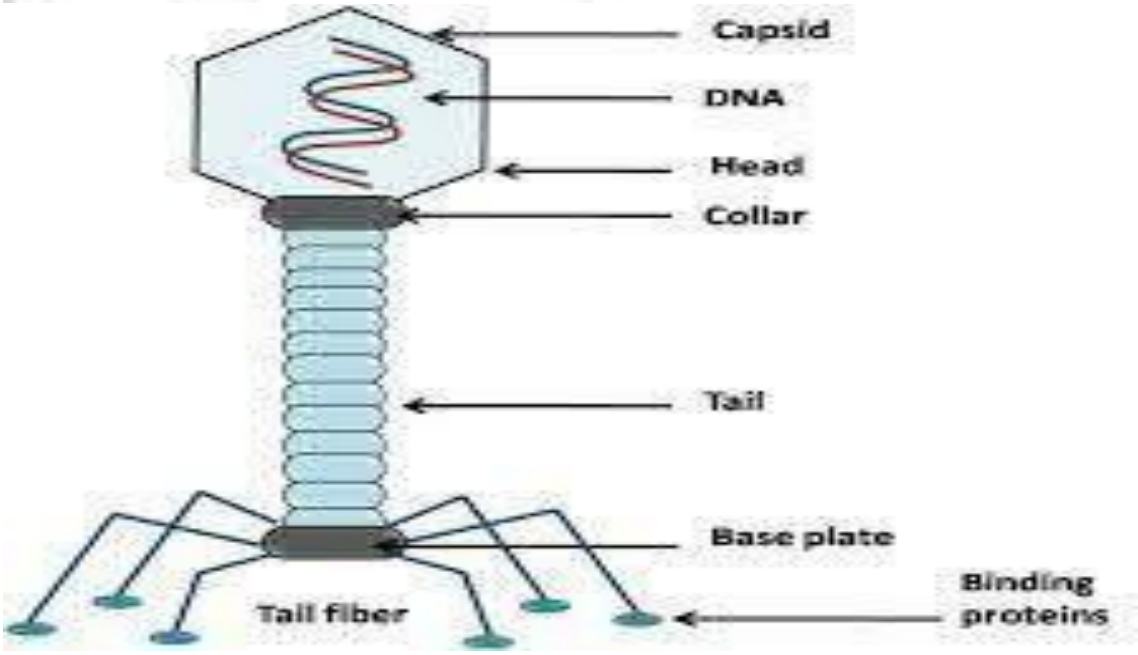
Bacteriophages or bacterial viruses are the viruses that infect bacteria and cause disease. These have great importance today in the study of microbial genetics and molecular biology. The bacteriophages are very large in size. This was discovered by Twort is 1915 and later studied by Herelle in 1977.

LAMBDA PHAGE

- Lambda phage is a bacterial virus, or bacteriophage, that infects the bacterial species *E. coli*.
- The lambda phage DNA is a linear and double stranded and at ends are single stranded which are complementary to each other.
- This produces repressor protein which resist lysis of cell.
- This virus is a temperate phage.
- It consists of lytic and lysogenic pathways.
- It comprises of head, neck and tail.
- The sticky ends are complementary to each other.
- When the linear DNA enclosed a protein body.
- Belongs to Siphoviridae family.
- Nearly 50 nm diameter.
- Icosahedral head.
- A flexible tubular protein tail.
- Connector serves as a site for attachment of performed head to tail.

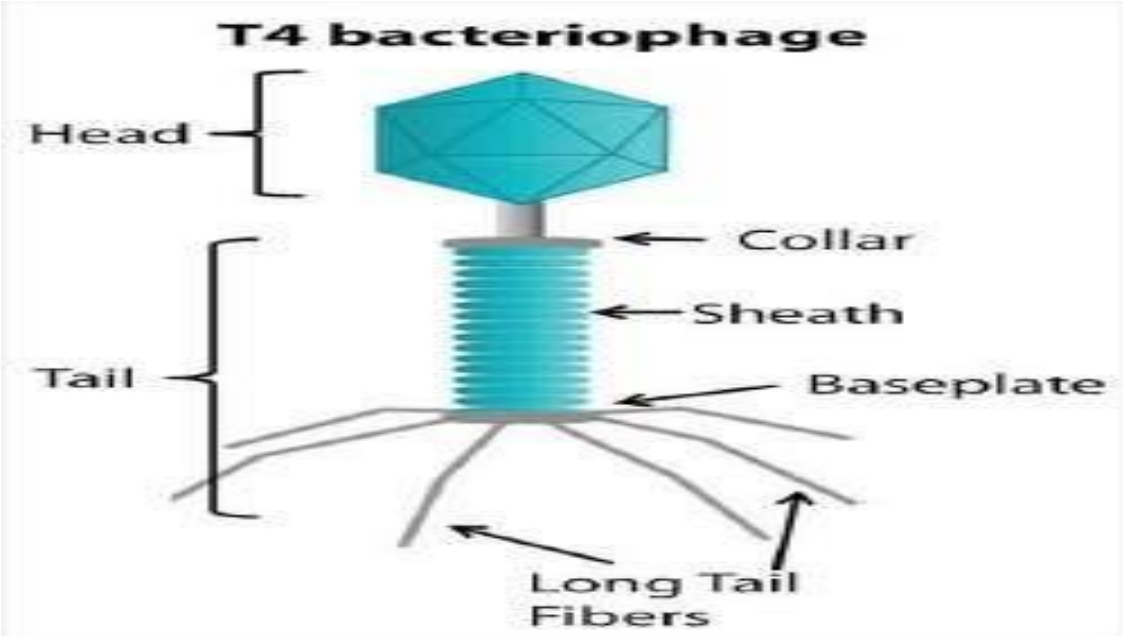
- It is a temperate phage.
- Contains large genome.





T4 PHAGE

- T4 is a bacteriophage that infects E. coli bacteria.
- T4 is among the largest phages.
- Complete genome sequence is 169-170 kbp long.
- About 80-100 nm wide.
- Contains head, collar and tail.
- Encodes about 300 genes.
- T4 biology and its genomic sequence provide the best- understood model for modern functional genomics and proteomics.
- Its tail fibres allow attachment to a host cell.
- The T4 tail is hollow so that it can pass its nucleic acid to the cell it is infecting during attachment.

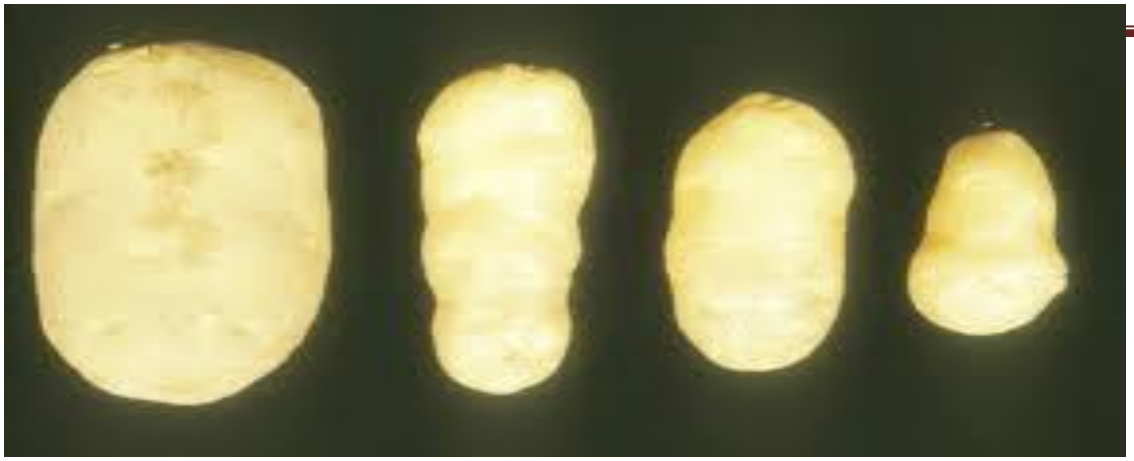


VIROID

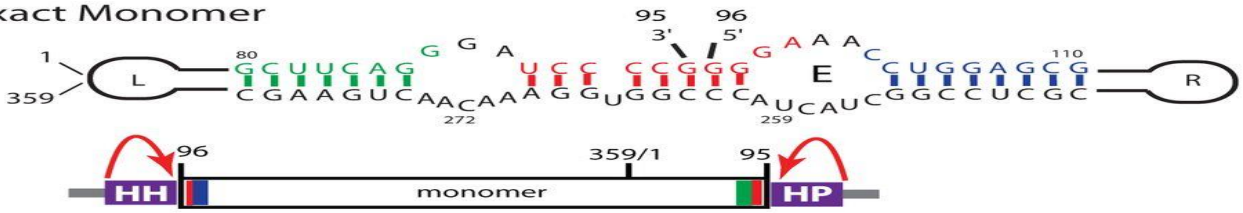
Viriods are the smallest infectious pathogens known. They are solely composed of a short strand of circular, single-stranded. RNA without protein coat. All known viriods are inhabitants of higher plants, in which most cause diseases, some of which are of slight to catastrophic economic importance.

The first recognized viroid, the pathogenic agent of the potato spindle tuber disease, was discovered, initially molecularly characterized and named by Theodor otto Diener, plant pathologist at the U.S. Department of Agriculture's Research Centre in Beltsville, Maryland 1971. This viroid is now called Poato Spindle Tuber Viriod, abbreviated PSTVD. Although viriods are composed of nucleic acid, they do not code for any protein.

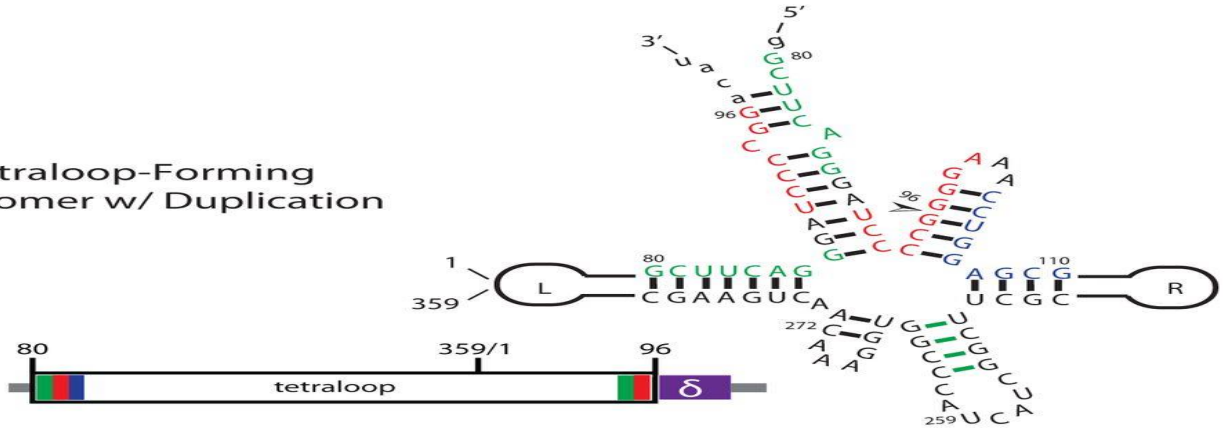
- POTATO SPINDLE TUBER VIROID- PSTV Symptoms range from mild to severe.
- Mild strains produce no obvious symptoms.
- Symptoms in severe strains are dependent on environmental conditions and are most severe in hot conditions.
- Symptoms may be mild in initial infections but become progressively becomes worse.
- Transmission through aphids and also mechanical transmissions.
- This was the first viroid to be identified.
- PSTV is a small, circular RNA molecule.
- All potatoes and tomatoes are susceptible to PSTV and there is no form of natural resistance.
- Prions are misfolded proteins.



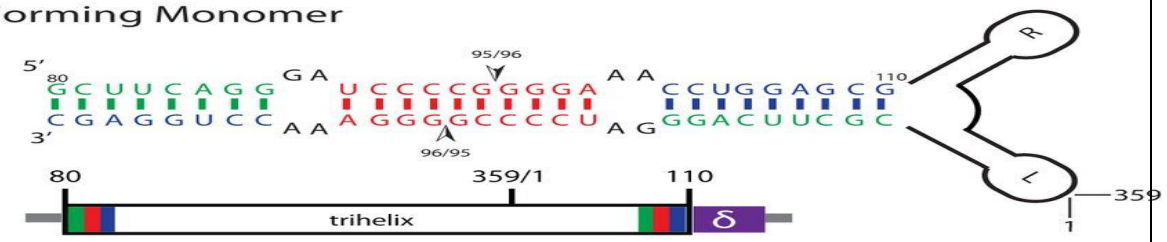
A. Exact Monomer



B. Tetraloop-Forming Monomer w/ Duplication



C. Trihelix-Forming Monomer



PRIONS

- Prions were first discovered by Stanley. B. Prusiner and his associates.
- According to them, prions are tiny organisations made entirely of proteins and contain no gene material of their own.
- Prions are 100-1000 times smaller than the smallest organisations.
- According to the reports these can survive heat, radiation and chemical treatments that normally destroy viruses.
- The infection effect of prions on the host varies from acute, chronic, persistent, latent and slow progressive tumor producing influence.
- These are capable of infecting even in the absence of nucleic acids.
- The mechanism of replication is still unknown.
- Prions have been implicated in the disease 'scrap' which is a fatal disease of nervous system of sheep.
- Prions have also been implicated in disease such as senile dementia, multiple sclerosis etc.
- Prions are an infectious agent composed entirely of protein material.
- Prion aggregates are extremely stable and accumulate in infected tissue, causing tissue damage and cell death.
- These are resistant to denaturation by physical and chemical agents.

CJD- CREUTZFELDT- JAKOB DISEASE

- CJD is an incurable and universally fatal neurodegenerative disease.
- CJD is also called a human form of mad cow disease (Bovine Spongiform Encephalopathy or BSE).
- CJD is caused by a transmissible agent called Prion.

- Inherited type of transmission.

- CJD causes the brain tissue to degenerate rapidly and as the disease destroys the brain, the brain develops holes and the textures changes to resemble that of kitchen sponge.

Types of CJD

- Variant CJD (vCJD) caused by consuming food contaminated with prions.
- Sporadic (sCJD), caused by a mutation arising in an individual. This accounts for 85% of cases of CJD.
- Familial (fCJD), caused by an inherited mutation. This accounts for majority of the other 15% cases of CJD.
- Medical procedures that are associated with the spread of this form includes blood transfusion from the infected person, use of huam derived pituitary growth hormones, gonadotropin hormone therapy etc.

KURU

- It is a very rare, incurable neurodegenerative disorder.
- Prevalent among the fore people of Papua New Guinea in 1950s and 60s.
- Kuru is a form of transmissible spongiform encephalopathy (TSE) caused by prion proteins.
- The term 'Kuru' derives from the fore word 'Kuria' or 'Guria' (to shake) due to the body tremors that are a classic symptoms of the disease and kuru itself means 'trembling'.
- The disease is more prevalent among women and child.
- Symptoms include body tremors, random outbursts of laughter, gradual loss of co- ordination.
- Complications include infection and pneumonia during the terminal stage.
- Risk factor may be coming into close contact with the brain of an infected individual.

D BSE- BOVINE SPONGIFORM ENCEPHALOPATHY

- BSE commonly known as Mad Cow Disease, is a fatal neurodegenerative disease (encephalopathy) in cattle that cause spongiform degeneration of brain and spinal cord.
- BSE has a long incubation period of 2.5 to 5 years.
- Signs are not seen immediately in cattle's, due to extreme long incubation period.
- BSE is caused by a misfolded protein- a prion.
- Symptoms include abnormal behavior, trouble walking and weight loss, hyper- responsiveness; later the cow becomes unable to move finally death.
- Cattles are believed to have been infected from being fed meat and bone meal.
- Diagnostic method may be suspected based on the symptoms, confirmed by examination of the brain.
- Cases are classified as classic or atypical types.
- These can replicate.
- In the United Kingdom, the country worst affected by an epidemic in 1986-1998, more than 1,80,000 cattle were infected and 4.4 million slaughtered during the eradication program.
- The disease may be transmitted to humans by eating food contaminated with the brain, spinal cord or digestive tract of infected carcasses.
- iagnostic method is by the neurological examination.

EUKARYOTIC MICROORGANISMS

Eukaryotes are organisms whose bodies are made up of eukaryotic cells, such as protists, fungi, plants and animals. Eukaryotic cells are cells that contain a nucleus and organelles, and are enclosed by a plasma membrane. Organisms with eukaryotic cells are grouped into the biological domain Eukaryota (also sometimes called Eukarya). The other two domains of life, Archaea and Bacteria, have prokaryotic cells, which are simpler and lack organelles except for ribosomes, which make proteins.

Types of Eukaryotes

There are four types of eukaryotes: animals, plants, fungi, and protists. Protists are a group of organisms defined as being eukaryotic but not animals, plants, or fungi; this group includes protozoa, slime molds, and some algae. Protists and fungi are usually unicellular, while animals and plants are multicellular. Unicellular eukaryotes can reproduce sexually or asexually. They move with the use of flagella, which are small thread-like appendages that extend from the cell membrane. Unicellular eukaryotes perform many of the same actions as multicellular eukaryotes, such as locomotion, respiration, digestion, excretion, and reproduction.

Fungi

A fungus (plural: fungi) is any member of the group of eukaryotic organisms that includes microorganisms such as yeasts and molds, as well as the more familiar mushrooms. These organisms are classified as a kingdom, which is separate from the other eukaryotic life kingdoms of plants and animals.

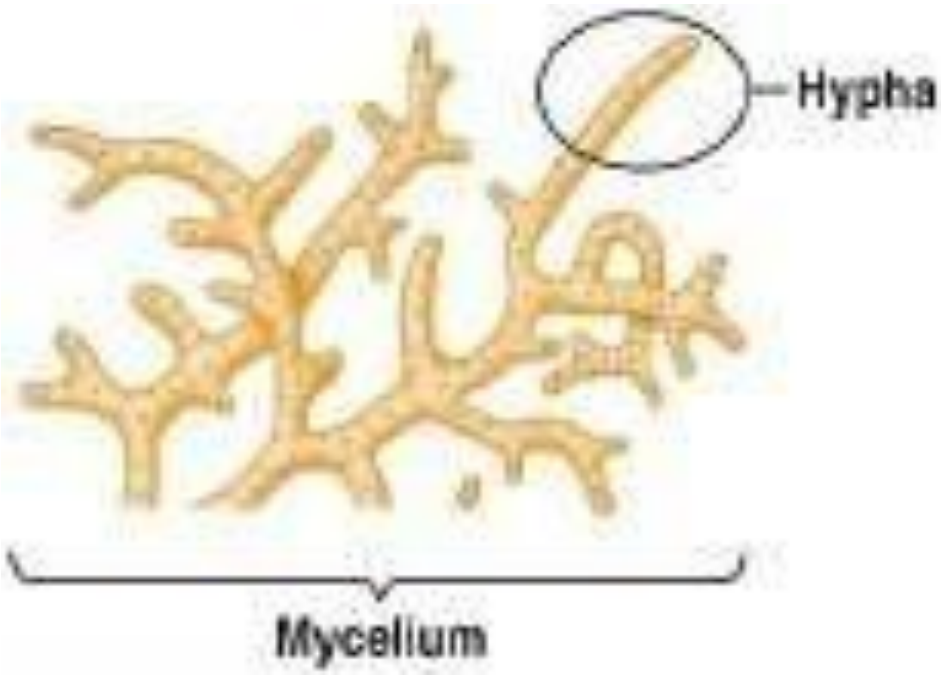
Characteristics of Fungi

1. Fungi is a separate kingdom. Fungi are Eukaryotic organism

2. More than 2,00,000 fungi species are known

3. Morphology:

- The plant body of true fungi (Eumycota), the plant body is a thallus.
- It may be non-mycelial or mycelial. The non-mycelial forms are unicellular; however, they may form a pseudomycelium by budding. In mycelial forms, the plant body is made up of thread like structures called hyphae
- Fungi exists in two fundamental forms, filamentous or hyphal form (MOLD) and singe celled or budding form (YEAST).



- But for the classification of fungi, they are studied as mold, yeast, yeast like fungi and dimorphic fungi.
- Yeast is Unicellular while Mold is multicellular and filamentous

4. Fungi lacks Chloroplast.

5. Mode of nutrition:

- Fungi are organotrophic heterotrophs.
- Mostly Fungi are saprophytic and some are Parasitic

6. Fungi grow best in acidic environment (tolerate acidic pH).

7. Fungi can tolerate high sugar concentration and dry condition

8. Most of the fungi are Obligate aerobes (molds) and few are facultative anaerobes (yeasts)

9. Optimum temperature of growth for most saprophytic fungi is 20-30 C while (30-37) C for parasitic fungi.

10. Growth rate of fungi is slower than that of bacteria.

11. Cell wall is composed of chitin

12. Cell membrane consists of ergosterol

13. Reproduction: both asexual (Axamorph) and sexual (Teliomorph) mode of reproduction

- Asexual methods: fragmentation, fsomatic budding, fission, asexual spore formation
- Sexual methods: gametic copulation, gamate-gametangium opulation, gametangium copulation, somatic copulation and Spermatization.

14. More than 100 fungi are responsible for human infection.

15. Some fungi shows mutualistic relationship with higher plants, eg Mycorrhiza is symbiotic associated with root of gymnosperm

Classification of fungi

Phycomycetes

- Members of phycomycetes are found in aquatic habitats and on decaying wood in moist and damp places or as obligate parasites on plants.
- The mycelium is aseptate and coenocytic,Asexual reproduction takes place by zoospores (motile) or by aplanospores (non-motile).
- These spores are endogeneously produced in sporangium.
- Zygospores are formed by fusion of two gametes
- Examples: Mucor, Rhizopus and Albugo (the parasitic fungi on mustard).

Ascomycetes:

- Sexual spore produced within a sac like structure called ascus.
- Sexual spore are called ascospore

- Asexual reproduction occurs by single celled or multi celled conidia
- Ascomycetes are also known as sac mycetes.
- Hyphae are generally septated
- Examples: *Saccharomyces, Arthroderma, Gibberella*

Basidiomycetes:

- Sexual spore are produced externally on a basidium
- Sexual spore are known as basidiospore
- Asexual reproduction occurs by budding, fragmentation or conidia formation
- They are commonly called as mushroom group
- Hyphae are generally septated
- Examples: *Amanita, Agaricus, Filobasidiella*

Zygomycetes:

- Sexual spore are known as Zygospore
- Zygospore is formed by fusion of two similar cell.
- Asexual reproduction occurs by sporangiospore
- Hypahe are generally aseptated.
    - Examples: Rhizopus, Mucor, Basidiobolus, Conidiobolus

Deuteromycetes:

- No sexual stage is present
- Deuteromycetes are also known as fungi imperfecti.

- Asexual reproduction occurs by means of conidia.
- Most of the human and animal pathogens are present in this class.
- Examples: *Candida, Cryptococcus, Trichophyton, Epidermophyton, Histoplasma*

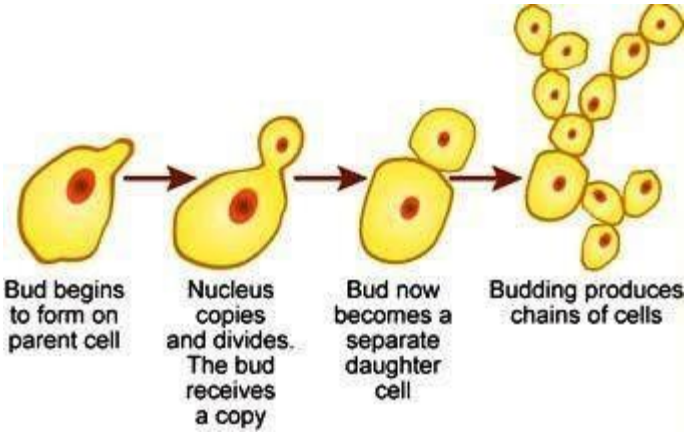
Reproduction

The fungi either reproduces vegetatively, asexually or sexually:

- Vegetative Reproduction

o Fragmentation: Some forms belonging to Ascomycotina and Basidiomycotina multiply by breakage of the mycelium.

o Budding: Some unicelled forms multiply by budding. A bud arises as a papilla on the parent cell and then after its enlargement separates into a completely independent entity.



o

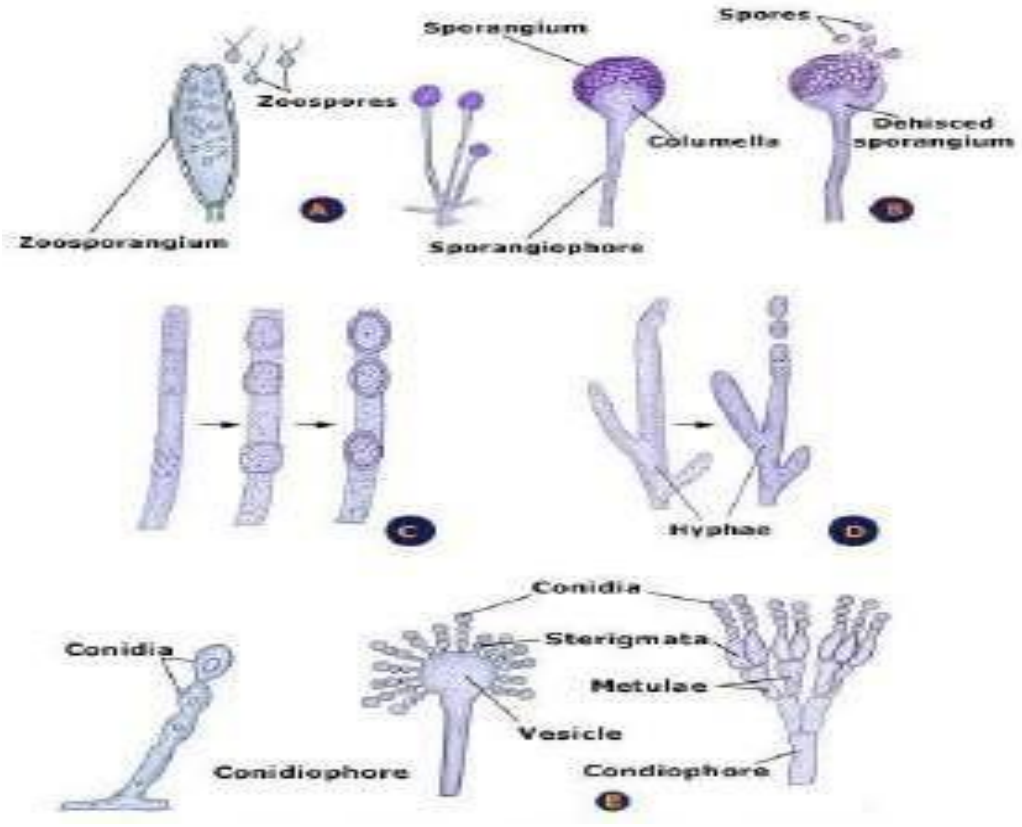
- Fission: A few unicelled forms like yeasts and slime moulds multiply by this process.

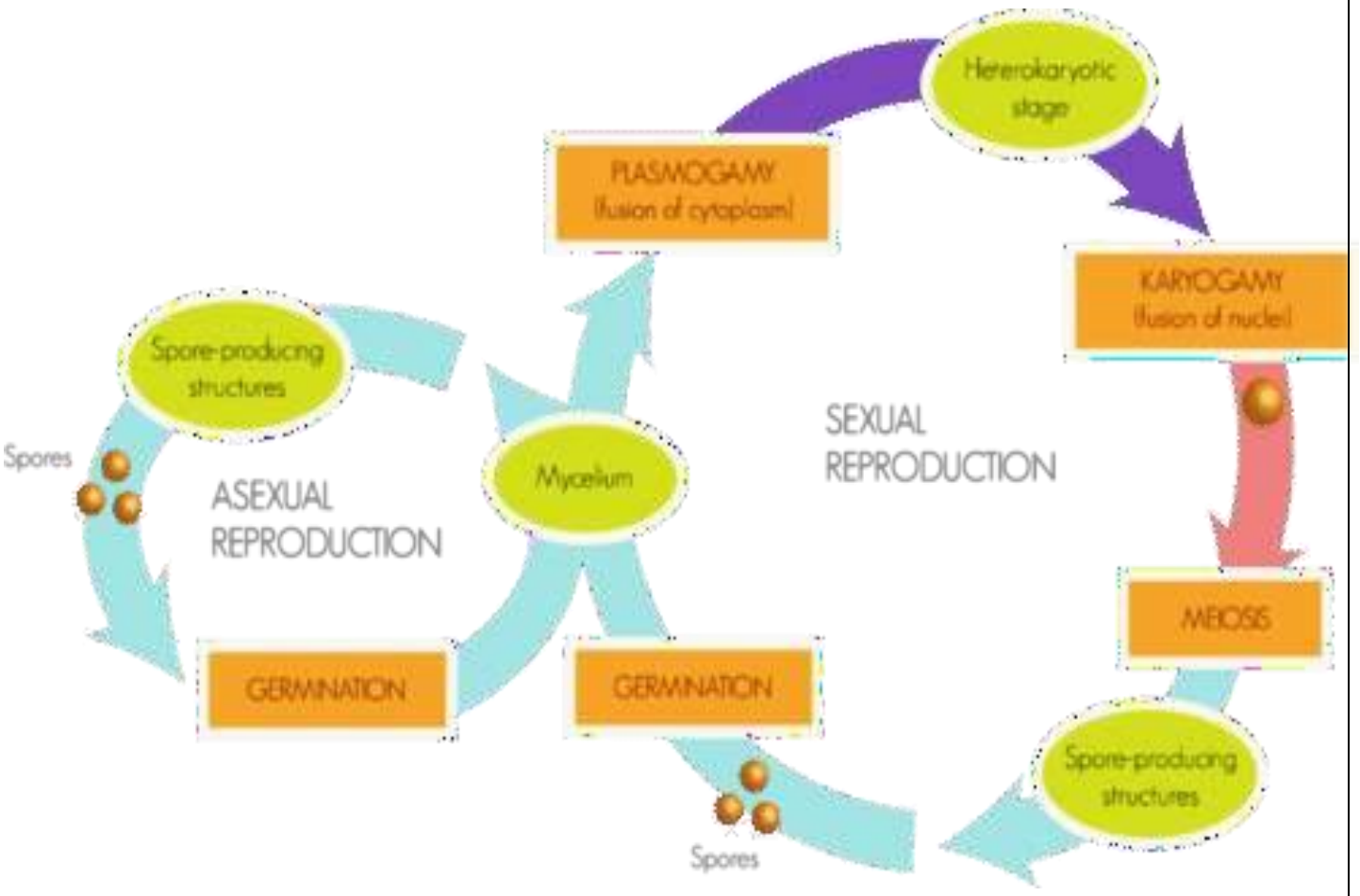
- Asexual Reproduction

- Sporangiospores: These are thin-walled, non-motile spores formed in a sporangium. They may be uni- or multinucleate. On account of their structure, they are also called as aplanospores.

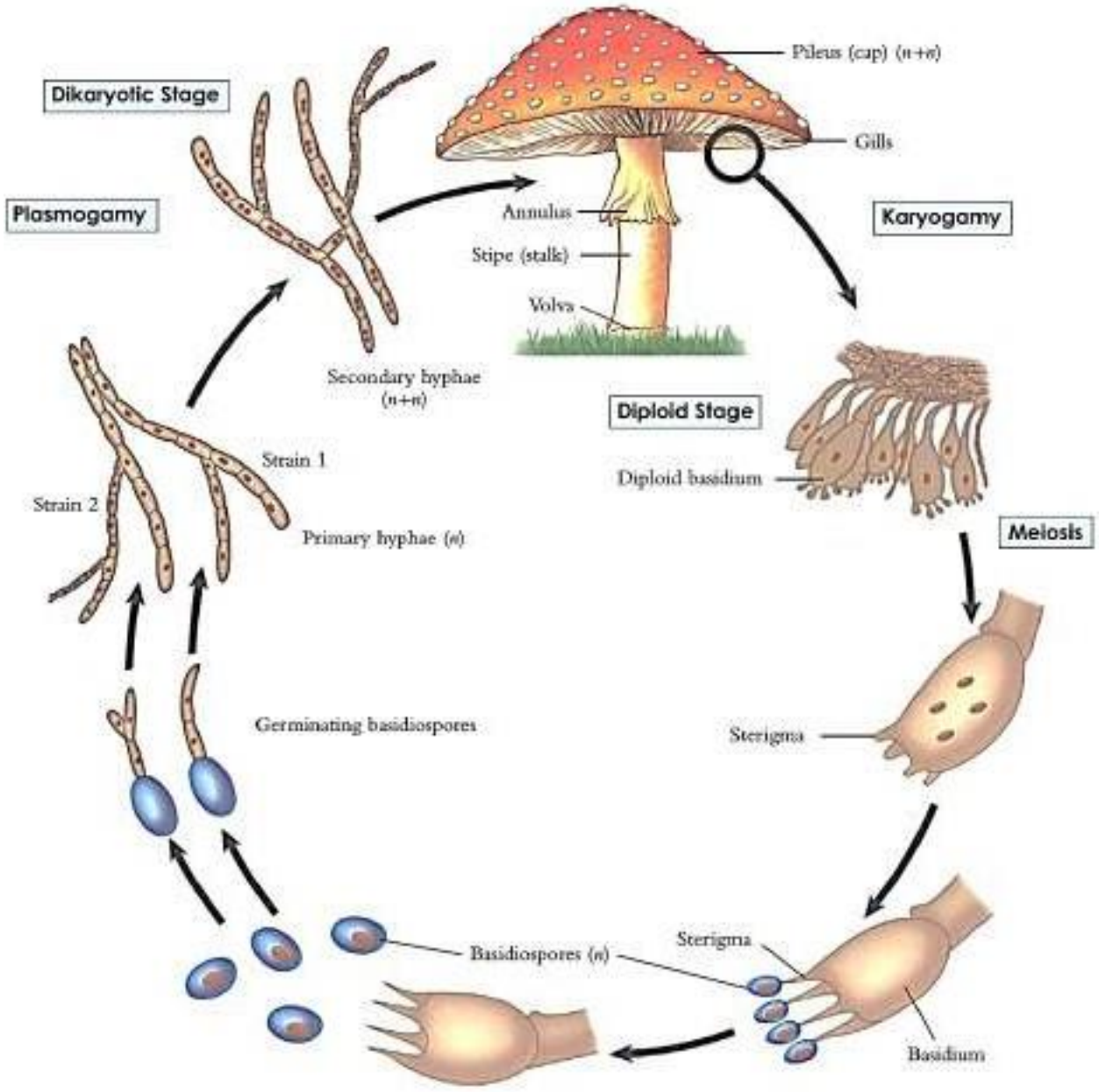
- Zoospores: They are thin-walled, motile spores formed in a zoosporangium.

- Conidia: In some fungi, the spores are not formed inside a sporangium. They are born freely on the tips of special branches called conidiophores. Thus, these spores are conidia.





- Sexual reproduction: With the exception of Deuteromycotina (Fungi imperfecti), we find sexual reproduction in all groups of fungi. During sexual reproduction, the compatible nuclei show a specific behaviour which is responsible for the onset of three distinct mycelial phases. The three phases of nuclear behaviour are as under:
  - Plasmogamy: Fusion of two protoplasts.
  - Karyogamy: Fusion of two nuclei.
  - Meiosis: The reduction division.


Dikaryotic Stage
Plasmogamy
Pileus (cap) (n+n)
Gills
Annulus
Stipe (stalk)
Volva
Karyogamy
Secondary hyphae (n+n)
Diploid Stage
Diploid basidium
Meiosis
Strain 1
Strain 2
Primary hyphae (n)
Germinating basidiospores
Sterigma
Basidiospores (n)
Sterigma
Basidium

Economic Importance Of Fungi

Fungi are an important organism in human life. They play an important role in medicine yielding antibiotics, in agriculture by maintaining soil fertility, are an important means of food, and forms the basis of many industries. Let us have a look at some of the fields where fungi are really important.

Importance in Human Life

Fungi are very important to humans at many levels. They are an important part of the nutrient cycle in the ecosystem. They also act as pesticides.

Biological Insecticides

Fungi are animal pathogens. Thus they help in controlling the population of pests. These fungi do not infect plants and animals. They attack specifically to some insects. The fungus *Beauveria bassiana* is a pesticide that is being tested to control the spread of emerald ash borer.

Reusing

These microbes along with bacteria bring about recycling of matter by decomposing dead matter of plants and excreta of animals in the soil, hence the reuse enriches the soil to make it fertile. The absence of activities of fungi can have an adverse effect on this on-going process by continuous assembly and piling of debris.

Importance in Medicine

- Metabolites of fungi are of great commercial importance.

- Antibiotics are the substances produced by fungi, useful for the treatment of diseases caused by pathogens. Antibiotics produced by actinomycetes and moulds inhibits the growth of other microbes.
- Apart from curing diseases, antibiotics are also used fed to animals for speedy growth and to improve meat quality. Antibiotics are used to preserve freshly produced meat for longer durations.
- Penicillin is a widely used antibiotic, lethal for the survival of microbes. The reason it is extensively used is since it has no effect on human cells but kills gram-positive bacteria.
- Streptomycin, another antibiotic is of great medicinal value. It is more powerful than Penicillin as it destroys gram-negative entities.
- Yield-soluble antibiotics are used to check the growth of yeasts and bacteria and in treating plant diseases.
- Administration of Griseofulvin results in the absorption by keratinized tissues and are used to treat fungal skin diseases(ringworms).
- Ergot is used in the medicine and the vet industry. It is also used to control bleeding post-child-birth.
- LSD – Lysergic acid, is a derivative of ergot and is used in the field of psychiatry.
- Consuming fungi called Clavatia prevents cancer of the stomach.

Importance in Agriculture

The fungi plant dynamic is essential in productivity of crops. Fungal activity in farmlands contributes to the growth of plants by about 70%.

Fungi are important in the process of humus formation as it brings about the degeneration of the plant and animal matter.

They are successively used in biological control of pests. Plant pests are used as insecticides to control activities of insects. For example – *Empausa sepulchralis*, *Cordyceps melonhae*.

Use of fungal pesticides can reduce environmental hazards by a great extent.

Fungi are also used in agricultural research. Some species of fungi are used in the detection of certain elements such as Copper and Arsenic in soil and in the production of enzymes. For instance, biological and genetic research on fungi named Neurospora led to the One Gene One Enzyme hypothesis.

The fungi live in a symbiotic relationship with the plant roots known as mycorrhiza. These are essential to enhance the productivity of farmland. 80-90% of trees could not survive without the fungal partner in the root system.

Importance in Food industry

Some fungi are used in food processing while some are directly consumed. For example –

Mushrooms, which are rich in proteins and minerals and low in fat.

Fungi constitute the basis in the baking and brewing industry. They bring

about fermentation of sugar by an enzyme called zymase producing alcohol which is used to make wine.

Carbon dioxide- a by-product in the process, is used as dry ice and also in the baking industry to make the dough (rising and lightening of dough).

Saccharomyces cerevisiae is an important ingredient in bread, a staple food of humans for several years. It is also known as the baker's yeast.

Algae

The term "algae" covers many different organisms capable of producing oxygen through photosynthesis (the process of harvesting light energy from the sun to generate carbohydrates). These organisms are not necessarily closely related. However, certain features unite them, while distinguishing them from the other major group of photosynthetic organisms: the land plants.

GENERAL CHARACTERSTICS OF ALGAE

1. Algae are the simplest multicellular plants. Some are unicellular eg. *Chlamydomonas*
2. Pant body: known as Thallus and they are avascular
3. Habitat: Algae are usually aquatic, either freshwater or marine and some are terresterial.
4. Algae are eukaryotic thallophytes.
5. Algae are photoautotrophs.
6. Storage form of food: Starch
7. Reproduction: Algae reproduce either by vegetative, asexual or sexual method
8. Vevetative method: fragmentation, hormogonia
9. Asexual spore: zoospores, aplanospores, hypnospores, akine

CLASSIFICATION OF ALGAE

On the basis of photosynthetic pigments algae classified into three classes.

1. Chlorophyceae (green algae)
2. Phaeophyceae (brown algae)
3. Rhodophyceae (red algae).

1. Chlorophyceae (Green algae) General characterstics of Chlorophyceae

- It is the largest class of algae
- They are commonly known as green Algae.
- Photosynthetic pigments: They possesses chlorophyll a, chlorophyll b and small amount of β-carotenoids.
- The chloroplasts shows various shape ie. Spiral shape in *Spirogyra*, cup shaped in *Chlamydomonas*, star shaped in *Zygnema*, girdle shaped in *Ulothrix*
- Habitat: Mostly freshwater (*Spirogyra*, *Oedogonium, Chlamydomonas*, *Volvox*, etc), some are marine (*Sargassum*, *Laminaria*, etc) and some are parasitic (*Polysiphonia, Harvevella, Cephaleuros*)
- Distribution: they are cosmopolitan in distribution
- They are unicellular as well as multicellular.
- Each cell is eukaryotic
- Thalllus: their body structure, shape and size varies.
- Examples: *Chlamydomonas:* unicellular free living
- *Volvox*: colonial form
- *Spirogyra:* multicellular, unbranched filamentous form

- *Ulva*: multicellular, parenchymatous form
- Storage form of food: Starch
- Pyrenoids stores starch
- Cell wall has two layer: outer layer composed of pectose and inner layer is composed of cellulose
- Reproduction: vegetative, asexual and sexual method
- Vegetative : fragmentation
- Asexual: asexual sopre (akinete, aplanospore, azygospore)

Sexual: isogamous, anisogamous, oogamous type gametic fusion

2. Phaeophyceae (Brown algae)

General characteristics of Phaeophyceae

- Pheophyceae are called commonly known as brown algae
- Photosynthetic pigments: They possesses brown colored photosynthetic pigments fucoxanthin and β-carotenoids in addition to chlorophyll a and c.
- Habitat: They are almost marine, very few are fresh water eg.
- Thallus: they are multicellular brown algae. No unicellular and colonial (motile or non-motile) brown algae till known.
- Storage form of food: laminarin starch, manitol (alcohol) and some store iodine also.
- Reproduction: vegetative, asexual and sexual methods
- Vegetative: fragmentation.
- Asexual: asexual spores (motile zoospores).

- Sexual: isogamous or oogamous type gametic fusion.

3. Rhodophyceae (Red algae)

General characteristics of Rhodophyceae

- Rhodophyceae are commonly known as Red Algae
- Photosynthetic pigments: They possesses Red colored photosynthetic pigments r- phycocyanin and r-phycoerythrin along with chlorophyll a, d, xanthophyll and β- carotenoid
- Habitat: They are aquatic, mostly marine. Some are freshwater

  e.g. *Batrachospermum*.
- Thallus: Red algae show a variety of life forms-
- Examples: Unicellular- *Porphyridium*,
- multicellular- *Goniotrichum*,
- Parenchymatous- *Porphyra*,
- unicellular colonies-*Chroothece*,
- Storage form of food: Floridean starch and floridosides sugar.
- Reproduction: vegetative, asexual and sexual mode
- Vegetative: fragmentation
- Asexual reproduction: non- motile spores(akinete, aplanospore, azygospore)
- sexual reproduction: Oogamous.
- Some species shows Alternation of generations in their life cycle.

Reproduction The

modes are: 1. Vegetative 2. Asexual 3. Sexual.

Mode # 1. Vegetative Reproduction:

In this type, any vegetative part of the thallus develops into new individual. It does not involve any spore formation and there is no alternation of generations. It is the most common method of reproduction in algae.

Mode # 2. Asexual Reproduction:

Asexual reproduction involves the formation of certain type of spores — either naked or newly walled. It is a process of rejuvenation of the protoplast without any sexual fusion. Each and every spore germinates into a new plant. In this method, there is no alternation of generations.

Mode # 3. Sexual Reproduction:

All algae except the members of the class Cyanophyceae reproduce sexually. During sexual reproduction gametes fuse to form zygote (Fig. 3.18). The new genetic set up can develop by the fusion of gametes coming from the different parents.

Economic Importance of Algae

1. Algae Constitute the Link of Food Chain
2. Algae is Useful in Fish Culture
3. Algae is Used for Recreational Purposes
4. Algae is Useful in Sewage Treatment Plants
5. Algae and Water Supplies
6. Algae as the Origin of Petroleum and Gas
7. Algae and Limestone Formation

8. Algae is Used in Space Research and Other Fundamental Studies

9. Algae is Used as Food

10. Algae is Used as Fodder

11. Algae is Used as Fertilizers

12. Algae is Used as Medicine

13. Industrial Utilization of Algae

Protozoa

- term Protozoa (From Greek, **protos** meaning **first**, **zoon** meaning **animals**) was given by **Goldfass**.
- According to five-kingdom classification system, protozoans belong to the phylum Protozoa of kingdom **Protista**.

General characteristics:

- The protozoans are minute, generally microscopic and eukaryotic organisms.
- They are the simplest and primitive of all the animals with very simple body organization, i.e. **Protoplasmic grade** of organization.
- They are **unicellular** organisms without tissues and organs.

1. Habit and habitat:

- They may either be **free-living** (inhabiting fresh water, salt water or damp places) or **parasitic** (living as ecto- or endoparasites). Some are **commensals** in habit.
- Body is either naked or covered by a **pellicle** (plasmalemma or theca or lorica).
- Protozoans are either **solitary** or **colonial**; in colonial forms, the individuals are alike and independent.

2. Cell structure:

- Body shape is variable; it may be spherical, oval, elongated or flattened.
- They are usually **asymmetrical** but *Giardia* is **bilaterally symmetrical**.
- The protoplasm is differentiated into outer **ectoplasm** and inner **endoplasm**.

- They may have one or more nuclei. Nucleus may be **monomorphic** or **dimorphic**, **vesicular** (e.g. ***Entamoeba***) or massive (e.g. ***Amoeba***).
- Vesicular nucleus is commonly spherical, oval or biconvex.
- Dimorphic nuclei are found in **Ciliata**, one larger **macronucleus** (with **trophochromatin**) and other small **micronucleus** (with **idiochromatin**).
- Locomotory organelles are either **pseudopodia**, **flagella**, **cilia** or none.

3. Life processes:

- There is **no physiological division of labor** and all the vital activities of life are performed by a single cell.

Nutrition may be **holozoic** (animal like), **holophytic** (plant like) **sporozoic** or **parasitic**.

*In ***Euglena,*** the mode of nutrition is mixotrophic (both holozoic and holophytic).

- Digestion takes place inside the food vacuoles, i.e. **intracellular**.
- Respiration and occurs by **diffusion** through general body surface.
- Excretion occurs through general body surface like respiration. They are **ammonotelic** (excrete nitrogenous waste product in the form of ammonia).
- In some forms, egestion occurs through a temporary opening in the ectoplasm or through permanent opening called **Cytopyge**.
- **Contractile vacuoles** perform **osmoregulation** in fresh water forms and also help in removing excretory products.

*Contractile vacuole is absent in marine and parasitic forms.

4. Reproduction:

- Reproduction is either sexual or asexual; **asexual binary reproduction** occurs by **fission, multiple fission, budding** or **sporulation** and **sexual reproduction** occurs by **gamete formation** or **conjugation**.
- Binary fission may be simple or transverse or longitudinal or oblique.
- Life cycle often exhibits alternation of generation, i.e. it includes asexual and sexual phases.
- **Encystment** usually occurs to protect the cell from the **unfavorable conditions** and it also helps in dispersal.

Classification

On the basis of locomotory organelles, phylum Protozoa has been divided into the following four classes.

Class: Flagellata or Mastigophora

- Usually free living but few are parasitic forms.
- One or more **flalgella** usually present for locomotion or food capturing or attachment or protection.
- Body is covered with a pellicle which provides a definite shape.
- Some forms are green due to the presence of **chloroplasts** (e.g. *Euglena*).
- Asexual reproduction occurs by longitudinal binary fission.
- Single nucleus present in a cell.
- e.g. *Volvox, Noctiluca, Trichomonas, Trypanosoma, Giardia, Leishmania* etc.

Class: Rhizopoda or Sarcodina

- Free living or endoparasite.
- Contractile vacuole may be present or absent.
- Locomotory organelles are pseudopodia which also help in food capturing.
- Body is without a pellicle, and has no fixed shape.
- Protoplasm is differentiated into ectoplasm and endoplasm.
- Single nucleus is found in the endoplasm.
- Nutrition is holozoic and parasitic in few forms.
- Reproduction takes place by fission
- e.g. *Amoeba, Entamoeba* etc.

Class: Spoprozoa

- They are exclusively **endoparasites**.
- Locomotory organelles are **absent**.
- Body is covered by a thick pellicle.
- Nutrition is **saprophytic** and contractile vacuole is **absent**.
- Sexual reproduction takes place by **gamete** or **spore formation**.
- e.g. *Monocystis, Plasmodium* etc.

Class: Ciliata

- They may be either free living or **endoparasite**.

- Nucleus may be one, two or many in number.
- Body organization is complex.
- Body shape and size is definite and is covered with a pellicle.
- **Cilia** are the locomotory organelles.
- They have a holozoic mode of nutrition.
- Small **micronucleus** is **reproductive** in function whereas large **macronucleus** is **vegetative** in function.
- Asexual reproduction occurs by transverse fission and sexual by **conjugation**.
- e.g. *Opalina, Nyctotherus, Balantidium, Paramecium* etc.

 Economic importance

The protozoa are useful in the following ways:

2. Food

Protozoa provide food for insect larvae, crustaceans and worms, which are taken by large animals like fishes, lobsters, clams, and crabs, which are eaten by man. Thus they form sources of food supply to man both directly and indirectly.

3. Symbiotic Protozoa

Certain protozoa like *Trichonympha* and *Colonymphya* etc. live in the gut of termites which help in the digestion of cellulose. The digested cellulose is utilized by the host.

4. Insect Control

Several protozoa control harmful insects by persisting their bodies.

5. Helpful in Sanitation

A large number of protozoa living in polluted water feed upon waste organic matters and thus purify it.

Many protozoa feed upon bacteria and play important role in the sanitary bettermant and keeping water safe for drinking {Kudo, 1947).

6. Industry

The skeletal deposits of marine protozoa (Foraminifera and Radiolaria) form oceanic ooze at the sea-bottom. About 30% of oceanic bed is covered with the *Globigerina* ooze, these skeletal deposits are put to many uses. Some are employed as filtering agents, others are made into chalk and still others are used for abrasives.

Characteristics of typical bacterial cell structures

Structure	Function(s)	Predominant chemical composition
Flagella	Swimming movement	Protein
Pili		
Sex pilus	Stabilizes mating bacteria during DNA transfer by conjugation	Protein
Common pili or fimbriae	Attachment to surfaces; protection against phagotrophic engulfment	Protein
Capsules (includes "slime layers" and glycocalyx)	Attachment to surfaces; protection against phagocytic engulfment, occasionally killing or digestion; reserve	Usually polysaccharide; occasionally polypeptide

	of nutrients or protection against desiccation	
Cell wall		
Gram-positive bacteria	Prevents osmotic lysis of cell protoplast and confers rigidity and shape on cells	Peptidoglycan (murein) complexed with teichoic acids
Gram-negative bacteria	Peptidoglycan prevents osmotic lysis and confers rigidity and shape; outer membrane is permeability barrier; proteins have various functions	Peptidoglycan (murein) surrounded by phospholipid protein-associated LPS and lipopolysaccharide "outer membrane"
Plasma membrane	Permeability barrier; transport of solutes; energy generation; location of numerous enzyme systems	Phospholipid and protein
Ribosomes	Sites of translation (protein synthesis)	RNA and protein
Inclusions	Often reserves of nutrients; additional specialized functions	Highly variable; carbohydrate, lipid, protein or inorganic
Chromosome	Genetic material of cell	DNA

Plasmid	Extrachromosomal genetic material	DNA

Summary

1.Bacteria are phylogenetically related group of unicellular prokaryotic organisms distinct from archeae

2. Archaea is phylogenetically related group of prokaryotes which are primitive and distinct from bacteria

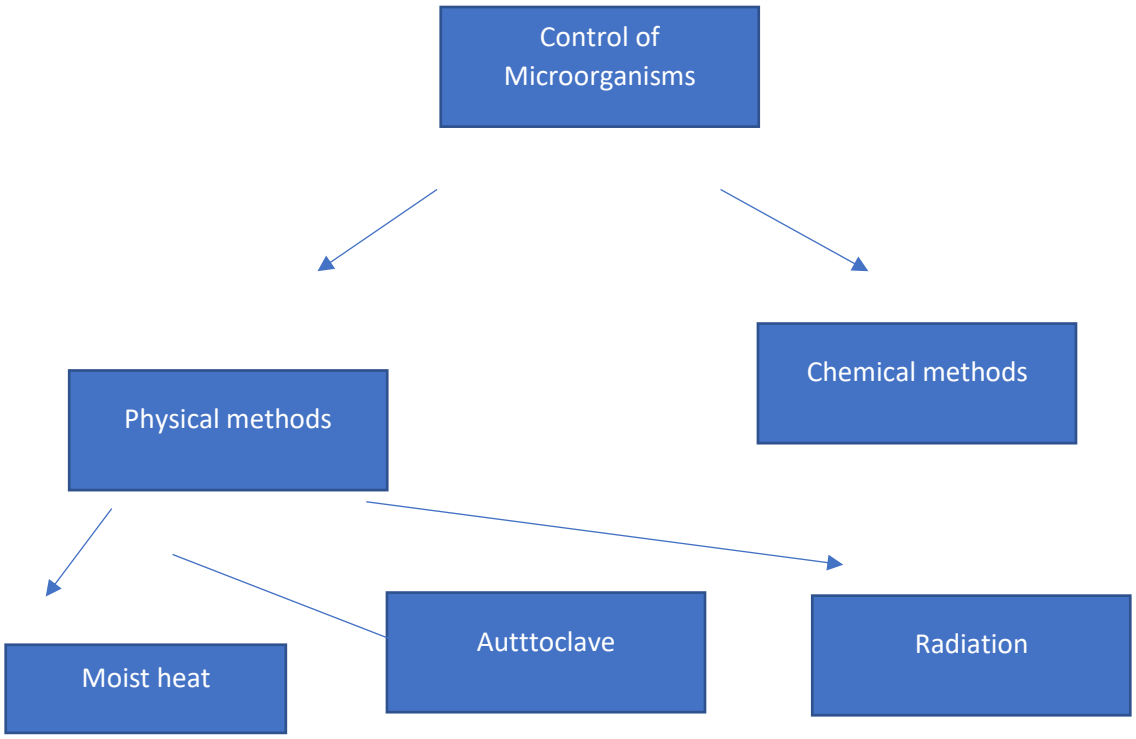
3. Fungi are group of eukaryotic organisms lacking chlorophyll. They range in size and shape from single celled yeast to multicellular mushrooms.

4. Algae refer the group of eukaryotic organisms with chlorophyll. They range in size and shape from single celled algae (Ex: Chlorella) to complex cellular structured plant like algae (Ex. Kelp) *5*

5. . Protozoa are group of eukaryotic organisms lack of cell wall. The morphology, nutrition and physiology is different from other groups

6. 6. Viruses are group of non-cellular organisms, parasite or pathogen to plant, animals and other microorganisms. They are too small and cab be visualized only under electron microscopes

Mind map


Control of Microorganisms
Physical methods
Chemical methods
Moist heat
Autttoclave
Radiation

UNIT-IV

MICROBIAL CONTROL MEASURES

Introduction

The control of microbial growth is most essential to limit the distribution of valuable nutrient sources and to prolong the life of perishable materials. Microbes are ubiquitous; Microbes grow and multiply under favourable conditions. In the laboratory culture technique they are allowed to grow and multiply for their study.

But under certain circumstances, it becomes necessary to destroy, remove or suppress the growth of microbes by practical methods as they cause contamination, diseases and decay and this is called microbial control of microbes is necessary in several situations.

- To prevent the transmission of pathogenic microbes and check the spread of the disease in plants animals including man.
- To prevent the contamination of pure cultures in scientific and medical laboratories.
- To prevent the inference by unwanted microbial contamination in industrial processes.
- To prevent the decomposition and spoilage of food and food products,
- To prevent the growth for research studies, so that one microbe is not mistaken for the others.

The microbes can be inhibited eliminated or killed by numerous physical chemical and other means. The agents which act against microbes are called "*antimicrobial agents*". Varieties of microbes differ greatly in their susceptibility to antimicrobial agents.

Many biological characteristics influence the rate at which the microbes are killed or inactivated by various antimicrobial agents. Many a time the choice of an antimicrobial agent depends on the type of microbe, (spores or growing vegetative cells). Its stage of growth (young or old), the environment in which it is present (air, water, soil, food, skin, sewage etc.).

It is prerequisite for one to know some common terms used in connection with microbial control.

Some of them are listed below.

ANTISEPTIC (ASEPTIC)

Acting against or opposing sepsis putrefaction or decay by either preventing or arresting the growth of microorganisms. Aseptic conditions are necessary in hospitals, dealing with patients, with communicable diseases and in microbiological laborator

Microbiology practical involves the culture of microbes in an aseptic condition and staining of microbes for their observation. Hence, the following 2 main types of techniques are developed.

1. Sterilization technique
2. Staining technique

STERILIZATION TECHNIQUE

Microbial culture requires 100%. Sterilization of glass wares, metal fools and culture media, which are used for microbial culture.

'The process by which an article, surface or medium is freed of all microbes either in vegetative state or in spore state. It is the most efficient method of killing all microbes from a given area, surface, object or material'.

Methods of sterilization

Sterilization of glass wares and culture media can be done by the following 3 principle methods.

1. Physical method
2. Chemical
3. Radiation

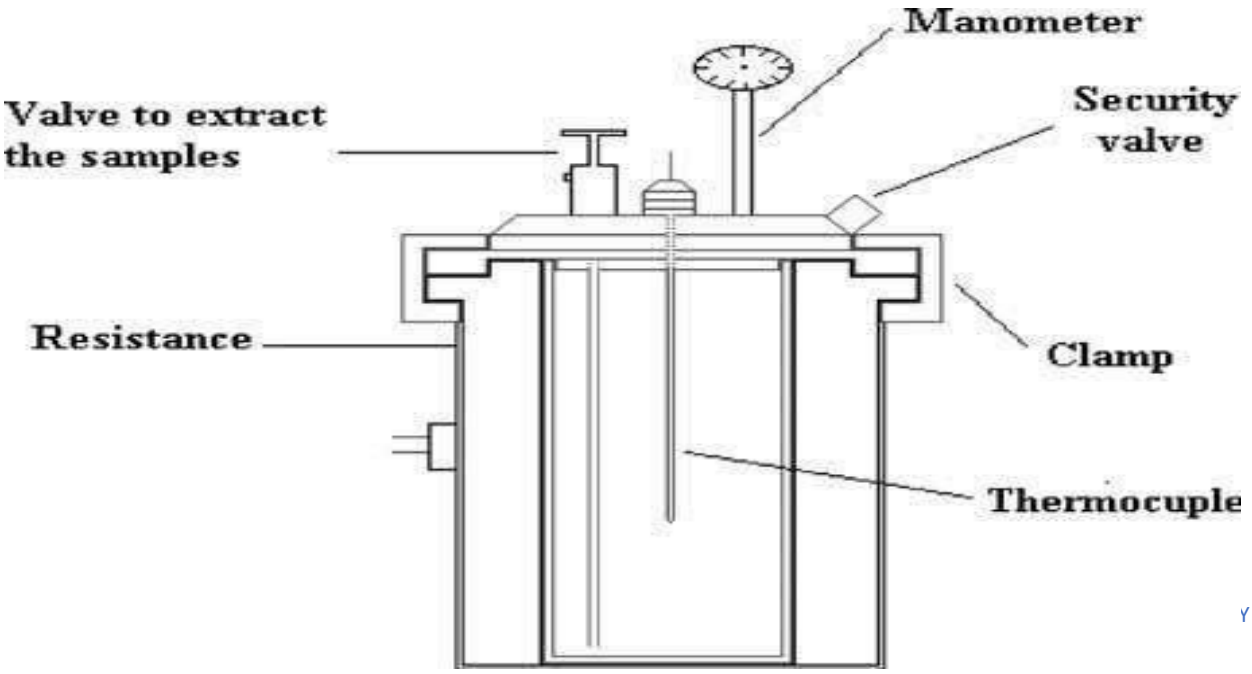
Physical method of sterilization: this include killing of microbes by applying moist heat as in steaming or dry heat as in hot air oven, or by filtration.

Application of physical methods of sterilization

- It is the most suitable method of sterilization for moisture sensitive material. Ex: oily substances and dry powder.
- It is suitable for assembled equipment providing sufficient time for penetration. Ex: all glass, syringes, test tubes etc.
- It is less damaging to glass and metal equipment than moist heat.
- This method is economically safe.

Sterilization of glass wares and culture media can be done by physical methods using.

1	Hot air oven method	3	Laminar air flow
2	Autoclave	4	Bacterial filters



AUTOCLAVE

It is an electrically operated instrumented for sterilization of glass ware and culture medium by most heat method or steam heat method.

Principle: when glass ware and culture media contaminated with microbes are exposed to moist heat or stem heat in the autoclave at 121° under 15 lb's for 15-20 min. moist heat kills the microbes by oxidising cellular components. This is the principle involved in sterilization of glass ware and culture medium by moist heat method or steam heat method.

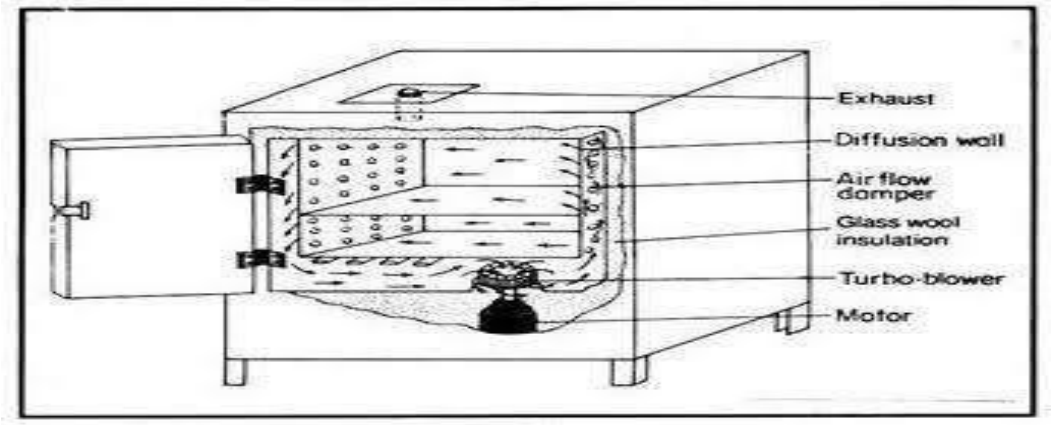
Working method

1. Cover all the glass ware and culture medium in the conical flask with newspaper separately, then keep them in autoclave and switch on the instrument.
2. Set the required temperature and required process by operating temperature setting knob and process setting knob.
3. Allow the instrument in working condition for 15 – 40 min. then switch off the instrument. Allow it to cool for room temperature.
4. Take out glass wares and culture media kept for sterilization and use them in microbial culture practicals.

Applications or uses

- Autoclave is used to sterilize the glass ware like beakers, conical flask, petridish and culture media.
- It is also used to sterilise used culture plates and tubes before their discard.

HOT AIR OVEN



It is an electrically operated heat isolated instrument used for sterilization of glass wares by dry heat method.

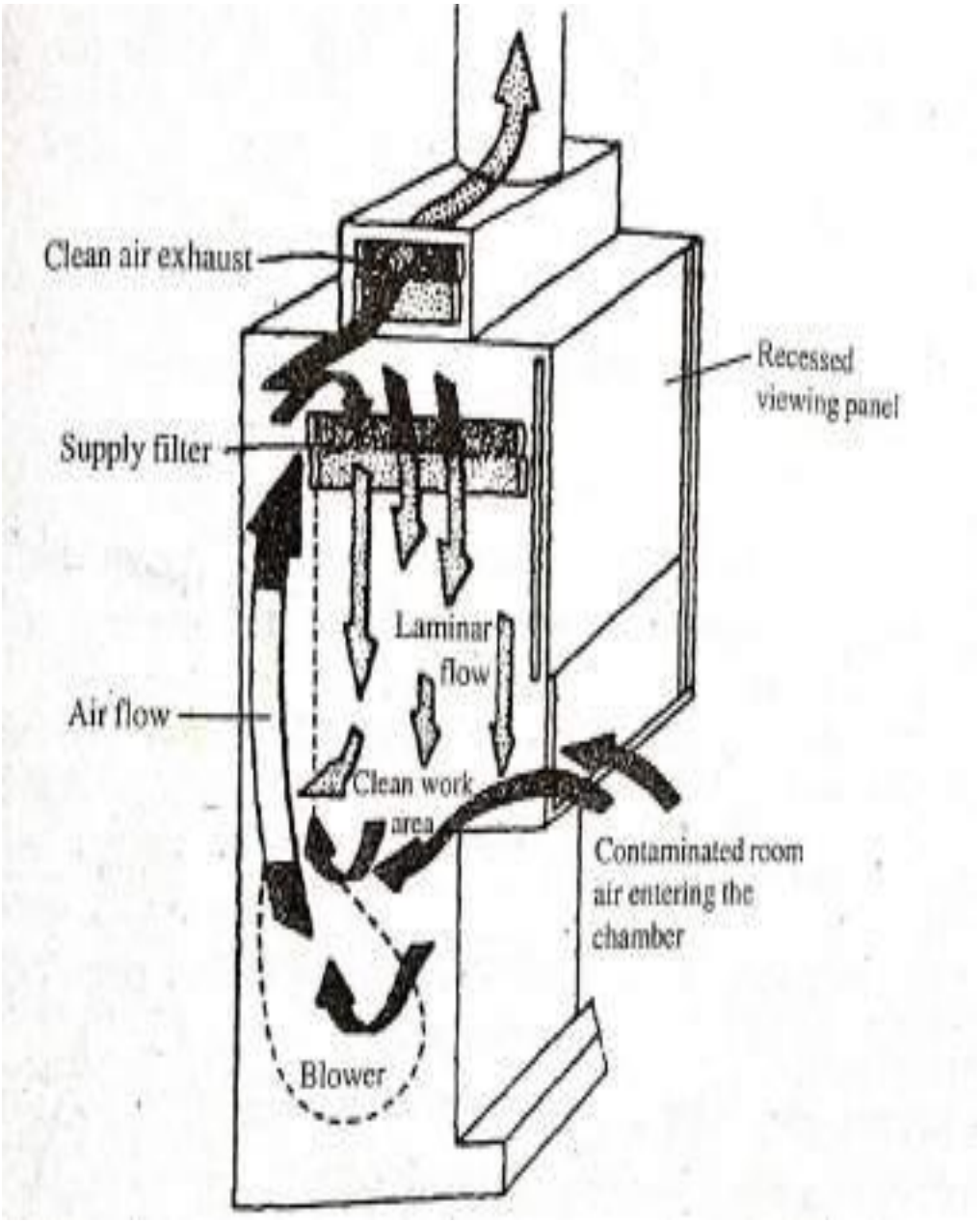
Principle: when glass wares contaminated with microbes are exposed to dry heat in the hot- air oven at 180° for 2-3 hours, dry heat kills the microbes present on the glass wares by oxidizing cellular components of microbes. This is the principle involved in sterilization of glass wares by dry heat method.

Working method:

1. Keep all the glass wares (beaker, pipette, conical flask, test tubes, petriplates etc.) which are to be sterilized inside the hot air oven.
2. Switch on the instrument set the temperature to 180° C by operating temperatures setting knob.
3. Allow the instrument in working condition face 2-3 hours. Then switch off the instrument allow it to cool for room temperature.
4. Then take out the glass ware kept for sterilization and use them for microbial culture practical.

pplication or uses

Hot air oven is used for sterilization of glass wares are beaker, conical flask, test-tube, petriplates etc by dry heat method.



LAMINAR AIR FLOW

Laminar air flow is defined as air moving at the same speed and in the same direction, with no or minimal cross-over of air streams (or 'lamina').

It is an electrically operated instrument used to create microbial free atmosphere required for inoculation process. In this instrument sterilization is done by filtration method and sterilization method.

Principle: when air contaminated with microbes passes through the HEPA (High Efficiency Particulate Air Filter) having pore size less than 1 micron, the entry of microbes along with the air through the

HEPA filter is prevented, this is the principle involved in creation of microbial free atmosphere in LAF (Laminar Air Flow).

When UV tube is on in LAF, UV rays also cause the sterilization by oxidising cellular component of the microbes. This is the principle also involved in sterilization of air in the LAF.

Application or uses

LAF is used for inoculation of microbial sample in to the culture media in the culture tube or culture plate under aseptic atmosphere.

Inoculation

The process of transfer of microbial sample with the help of inoculation loop into the culture media in the culture tube or culture plate is known as inoculation.

BACTERIAL FILTERS

Sterilization of liquid culture media, blood serum, water, which are to be used for bacterial culture practical, can be done by filtration method using bacterial filters.

Principle:

Bacterial filters are having pore size less than the bacterial cell size (less than 1 micron), when liquid culture media are blood serum or water contaminated with bacteria passes through the bacterial filters, the flow of bacteria through the pores will be prevented. This is the principle involved in sterilization by filtration method.

Kinds of bacterial filters

Bacterial filters include the following 3 types:

1. Seitz filter
2. Sintered glass filter
3. Membrane filter

SEITZ FILTER

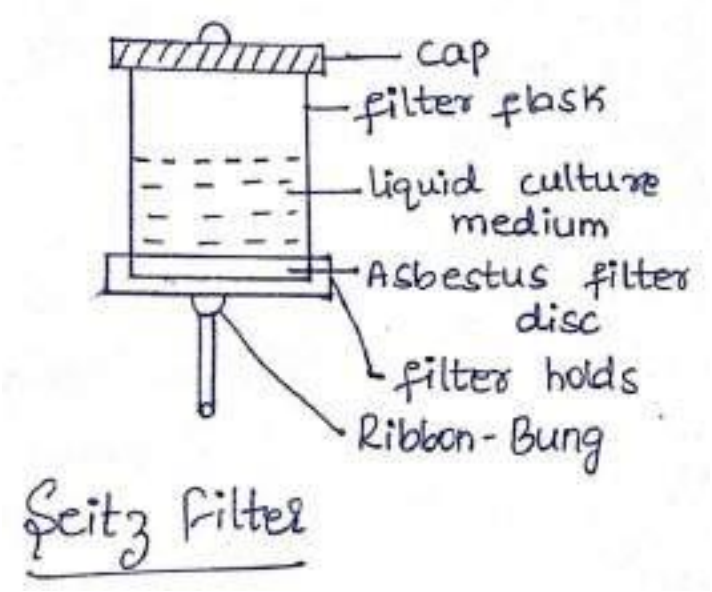
Seitz filter (Asbestos filter), is a kind of bacterial filters used for sterilization of liquid culture medium. It consists of cylindrical filter flask with screw cap at the top and filter holds at the base.

It consists of sheets or discs composed of compressed asbestos filter. The filters are 2-6 mm in thickness. They contain washed asbestos fibres, cellulae and some alkaline earth metals, such as magnesium compounds. The thickness of asbestos fibres and the gap between them determines the efficiency of the filter. These filter sheets are discs are clamped in a metal holder and either a negative or positive pressure is applied. Seitz filters are very soft and can be easily damaged by even rough handling.

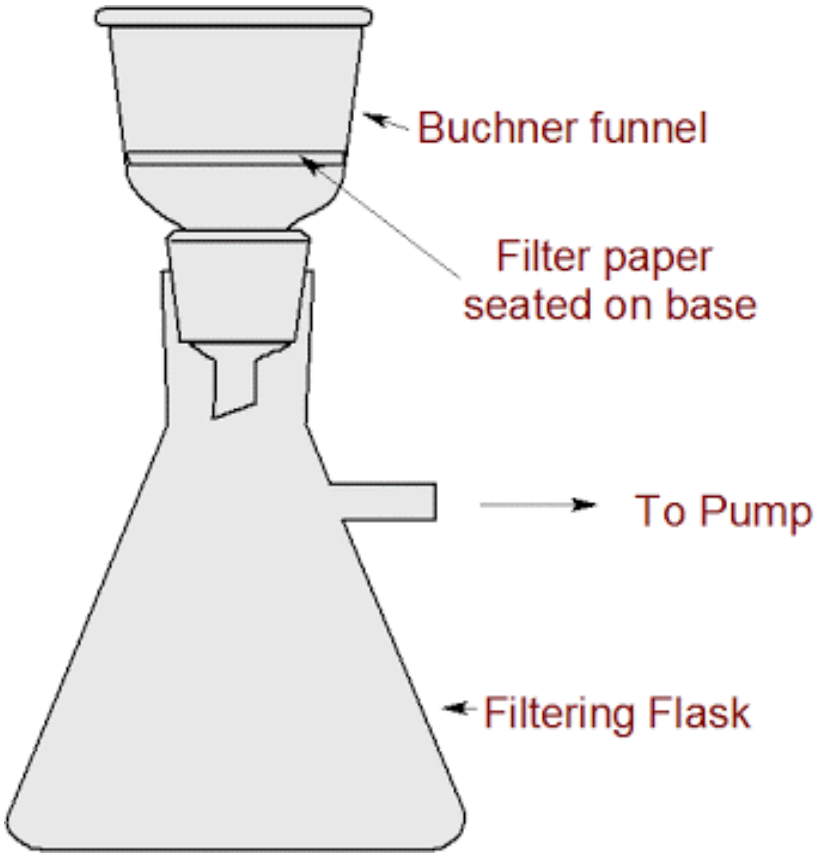
Applications

It is used for sterilization of liquid culture medium, blood serum, water by filtration method.

SINTERED GLASS FILTER



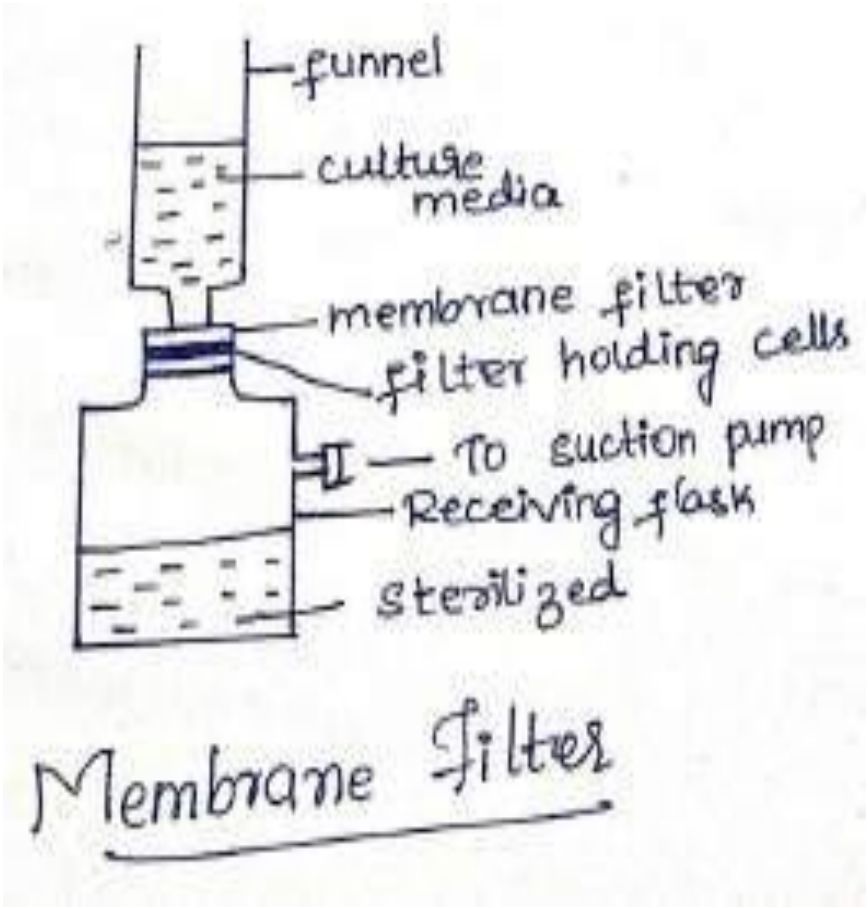
Sintered glass filters (Fritted glass filters) [Morton filters] It is prepared by heating finely pulverized glass in and fusing it in disc form in a suitable mold at the temperature just below its melting point. The discs are fused in to Buchner type or pyrex glass funnels



Either by a rubber stopper or through a ground glass joint.

The fitted glass filters are designed in several porosities, these may be classified in to extra course, course, medium, fine and ultra-fine in the order of decreasing porosity, and sintered glass filters are useful for small volume preparation.

Sintered glass filte



Application or uses

Sintered glass filter has less absorption property for microbes and chemicals. Hence, it is very easy to clean for its reused.

MEMBRANE FILTER (Ultra filters)

It is a kind of bacterial filter used to separate microbes present in water are liquid culture medium. It is made up of cellulose acetate filter disc.

Membrane filter unit consists of funnel over the receiving bottle. Between the funnel and receiving flask cellulose acetate membrane filter disc is mounted in the filter holding device. Receiving flask is connected between suction pump.

Advantages or Uses of membrane filters

- Bacteria are removed by sieving.
- Because the membranes used are very thin so absorption of medicament is negligible.
- A new disc is used for every disk of operation.
- Filtration is quite rapid.
- They do not liberate particles or chemical substances to filtrate.
- It is used to sterilize water or liquid culture medium used for microbial culture practical.

Merits

It serves as not only bacterial filter but also helps to identify types of bacteria present in the water sample or culture media. This involves the culture of membrane one disc changed with microbes in the nutrient agar culture medium.

RADIATION METHODS

Sterilization by radiation method

Sterilization of glass wares can also done by radiation method. Sterilization by radiations is called cold sterilization, because without boiling or heating microbes can be destroyed by radiation.

Energy transmitted from alpha, beta, gamma and X-rays and UV rays in the form of radiations. Based on mode of action on the chemicals radiations are classified into the following 2 groups:

1. **Ionizing radiations:** radiations which penetrate into the chemical molecule and cause the ionisation of atoms into the ions are called ionising radiations. Ex: radiations transmitted by alpha, beta, X rays.

   Effects:

a. Ionising radiations penetrates into the bacterial cell and causes the ionisation of atoms of DNA molecule.

b. These results in death of microbe.

2. **Non-ionising radiations**: radiations which never penetrate into the chemical molecule and never cause the ionisation of atoms into the ions are called non ionising radiations. Ex: radiations transmitted by light rays of shorter wave length and ultraviolet rays.

   Effects:

   Non ionizing radiations penetrates into the bacterial cell and never causes the ionisations of atoms, however, they causes damages in a DNA molecule in the cell. This results in death of the microbes.

UV RAYS

- Ultraviolet rays are widely used for sterilization purposes.

  The ultraviolet rays with wavelength around 2650 Å have potent bactericidal capacity. At this wavelength

- it is toxic to both spores and the vegetative cells.
- Many germicidal lamps are available that can emit high concentration of UV light in the most effective regions 260 nm - 270 nm. In these lamps UV light is generated by passing electricity through vaporised mercury in quartz tubes.

The UV-light effectively reduces microbial populations in the air of closed rooms or morgue, pharmacy, toilet facility, hospital operating rooms, In aseptic filling rooms the

pharmaceutical industry food and dairy industry the UV light is used for treatment of contaminated surfaces.

Since the UV-light has very light penetration power. Even a thin layer of glass filters off large amount of light. Hence only UV light can be used to destroy the microbes on the surface of an object.

Mechanism of action

Ultraviolet light is absorbed by many cellular materials, but most significantly the cellular DNA and induce the production of thyamine dimers, in which two adjacent thyamines become bonded. Such dimers interfere with proceedings of DNA replication.

The damage caused by UV light can be removed by specific intracellular enzymes by subjecting these to visible light in a process known as *photo reactivation,* The reaction is catalysed by the light dependent photolyase.

GAMMA RAYS

- Gamma radiations are high energy radiations emitted from certain radioactive isotopes such as $^{60}C_0$.
- Gamma rays have high energy short wavelength and high penetration power in to the biological tissues and are highly lethal to the DNA and other vital constituents and cause biological damage by produce

hyperactive free radicals like superoxides hydroxyl ions and peroxides.

- Because of their high penetration power and microbicidal effect gamma rays are attractive for use in commercial sterilization of bulk materials such as packaged food and medical devices.
- However certain practical problems must be resolved before gamma rays could be used for large scale uses.
- The design of the equipment should be such that it should not prove to be hazardous to the user.

CHEMICAL METHOD OF STERILIZATION

Sterilization of glass wares and instruments used in the microbial practical's can be done by using certain chemicals such chemicals are called sterilants.

Chemical sterilants are classified into following 2 groups.

1. **Dis-infectants**: chemical sterilants which prevent the spreading of microbes are called disinfectant. Ex: formaldehyde, phenol.
   - Disinfectants are mainly used for the sterilizing the surfaces used for an aseptic work.
   - In emergency they may be used for sterilizing the surgical instruments like forceps, scissors, knives, blades etc.
   - For this reason the acquired instruments need to wash with sterilized water.
   - The commonly used disinfectants include alcohol, iodine, chlorine etc.
2. **Antiseptics:** chemical sterilants which prevent the infection of wounds by microbes are called antiseptics. Ex: alcohol, iodine.

Mode of action of sterilants on microbes

Sterilants show the following effects on microbes.

1. Disinfection of cell wall and cell membrane of the microbes.
2. Dehydration of the microbes.

3. Coagulation of protein content of the microbes.
4. Defunctioning of enzymes in the microbes.

ALCOHOL

It is an organic solvent used as both disinfectant and antiseptic.

Ethyl alcohol in concentrations between 50-90% is effective against vegetative cells. However its maximum germicidal efficiency is exhibited in a concentration of 70% by weight.

Methyl alcohol (methanol) another alcohol is less efficient than ethanol, but it is not used as it is highly poisonous and cause harm to the users [methanol fumes can cause permanent damage to eyes]. Alcohols are mild disinfectants for practical application [70%] and are non- toxic when used for external application as antiseptics. They are effective in reducing the microbial flora of the skin and for the disinfection of clinical (oral) thermometers. 60% alcohol concentrations are effective against viruses.

Mode of action

Alcohols are protein denaturants that accounts for antimicrobial activity. It acts as dehydrating agent. As the alcohols are solvents for lipids and hence they can damage to the membrane lipids.

Effects on microbes

1. Disinfection of cell wall and cell membrane.
2. Dehydration of cells.
3. Coagulation of protein content of the cell.

ALDEHYDES

These are organic compounds with CHO group and used as only disinfectant. Ex: formaldehyde, glutaraldehyde.

Formaldehyde is one of the oldest known disinfectants. Formaldehyde and glutaraldehyde are the well-known sporicidal agents. Formaldehyde (HCHO) is a gas that is stable at high concentrations and at elevated temperatures. Commercially formaldehyde is marked in aqueous solution as formalin that contains 37-40% formaldehyde.

Practical applications: In aqueous solutions it is potent bactericidal and sporicidal also has lethal effect viruses. Formaldehyde in gaseous form (formalin) is chiefly used in disinfection and sterilization of closed area. Humidity and temperature have profound effect on the microbicidal action of formaldehyde.

Formalin is used to preserve the anatomical specimens. However one of the disadvantages that make the use of formaldehyde less frequently is that they are irritating to tissues and eyes.

Mode of action: Formaldehyde readily combines with the vital biomolecules such as proteins and nucleic acids and destroys them causing lethal effect on cells.

Glutaraldehyde is a saturated dialdehyde: It is effective against bacteria, viruses, fungi and spores of bacterial and fungi. It is used for sterilization of urological instruments, lensed instruments and respiratory therapy equipments.

Effects:

1. Denature of the cell
2. Coagulation of proteins of the cell.

PHENOLS

In 1870's Joseph Lister a surgeon used phenol to reduce infection of surgical incision and wounds. Phenol however is rarely used in recent days as it is very expensive. Hence less expensive and more effective phenolic derivatives are commonly used these days. A 5% of phenol rapidly kills the vegetative cells of microbes. Such derivatives include cresols, hexachlorophene, pentachlorophenol.

Practical uses: Phenol and its derivatives may be cither bactericidal or bacteriostatic, depending upon the concentrations used. The spores are much resist to the phenol.

The emulsions of phenols have increased germicidal property. The phenolic derivatives like have property to reduce the surface tension in addition to germicidal property. The solution of hexyl orcinol in water and glycerine has potent microbicidal property and used as an antiseptic in mouth wash, gargles-cough drops etc. Hexachloropene is a good skin antiseptic as it is surfactant. One of the widely used phenolic derivatives is the O-phenyl phenol. These are used in combination with soaps and hand wash.

Mode of action phenols

Phenols have variety of microbicidal effect; depending upon the concentration of the phenolic

compound and the duration of exposure the phenols solubilizes the lipids of cell membrane causing the cell contents to leak out. They also precipitate the proteins and denature the enzymes.

HALOGENS

Halogens (iodine, chlorine, bromine and fluorine] are potent germicides. Either in their free or in combined states.

- ***Iodine***

It is one of the older and most effective germicidal agents. Iodine is pungent dark brown chemical having metallic lustre. The iodine is traditionally used as a local antiseptic in households to treat wounds, cuts and scratches [2% iodine, 2% KI in 90% alcohol]. The iodine is also used in the form of "iodophores" that are mixtures of iodine with surfactant organic compounds that act as carriers and solubalizers for the iodine. Example, betadine, wesiodyne

Practical applications: It is highly bactericidal and sporicidal agent. Aqueous solutions of iodine are chiefly used for the disinfection of the skin, sanitization of food utensils and disinfection of air and water.

Mode of action: iodine is a strong oxidizing agent that irreversibly oxidizes some important functional groups metabolic pathways. They also inactive many enzyme by binding to active site amino acids like tyrosine residues.

- ***Chlorine***

It is the most widely used disinfectant either in gaseous form or in chemical combination with others compressed gas of chlorine is universally employed for the purification of municipal water supplies gaseous chlorine is used in large scale operations such as water purification plants as it requires a special equipment to handle. For the convenient use chlorine compounds than can be handled easily are hypochlorite.

(E.g.: Sodium hypochlorite or calcium hypochlorite, (chlorinated lime)).

Chloramines are organic chlorine compounds where one or more hydrogen atoms in imino group are replaced by the chlorine. They are used as sanitizing agent's disinfectants, or antiseptics.

Eg. Azochloramine, Dichloramine.

Applications

Chlorine compounds are widely used to control microbes and thus in water treatment, in the food industry, for domestic uses and in medicine, chlorine has wide spectrum of activity against viruses. The calcium hypochlorite is used in sanitizing, utensils, dairy equipments 1% solution of calcium hypochlorite is used in personal hygiene and as household disinfectant. Higher percentages of 5-12% are used as sanitizing agents in dairy and food industry.

GASEOUS AGENTS

The application of gaseous agents to control microbe has been practiced since a long time. Certain routinely used medical devices such as plastic syringes, blood transfusion apparatus and catheterization equipment's can be efficiently sterilized by the gaseous agents. The routinely used laboratory wares like plastic pipettes, petridishes and other equipments can be sterilized.

etc.

- Many gases like sulphur di oxide, chlorine, ozone, formaldehyde, β-propiolactone and ethylene oxide have bacterial effects.
- Nowadays ethylene oxide has become the most widely used gaseous sterilizing agent in pharmacy and medicine.

➢ *Ethylene oxide*

- It is a colourless gas at ordinary temperature.
- The mode of action of ethylene oxide to kill the microorganisms based on the process of alkylation of essential substances present in a protein molecule.

➢ *Formaldehyde*

- Formaldehyde gas is used for sterilization is produced by heating formalin to a temperature of 70-75°C with steam.
- Formaldehyde has a similar toxicity to ethylene oxide.
- This has been used commonly for fumigating the rooms and blankets in the hospital.

➢ *Disinfectants*

Application of sterilization by gaseous agents

- It sterilizes surgical instruments such as catheters, needles etc.
- Polythene bags can be sterilized by it.
- It is used for fumigating the room.
- It kills bacteria and all bacterial spores.
- It is used in alcoholic solution.
- It sterilizes the laboratory.

- They also used in disinfectants include alcohol, iodine, chlorine etc.

ummary

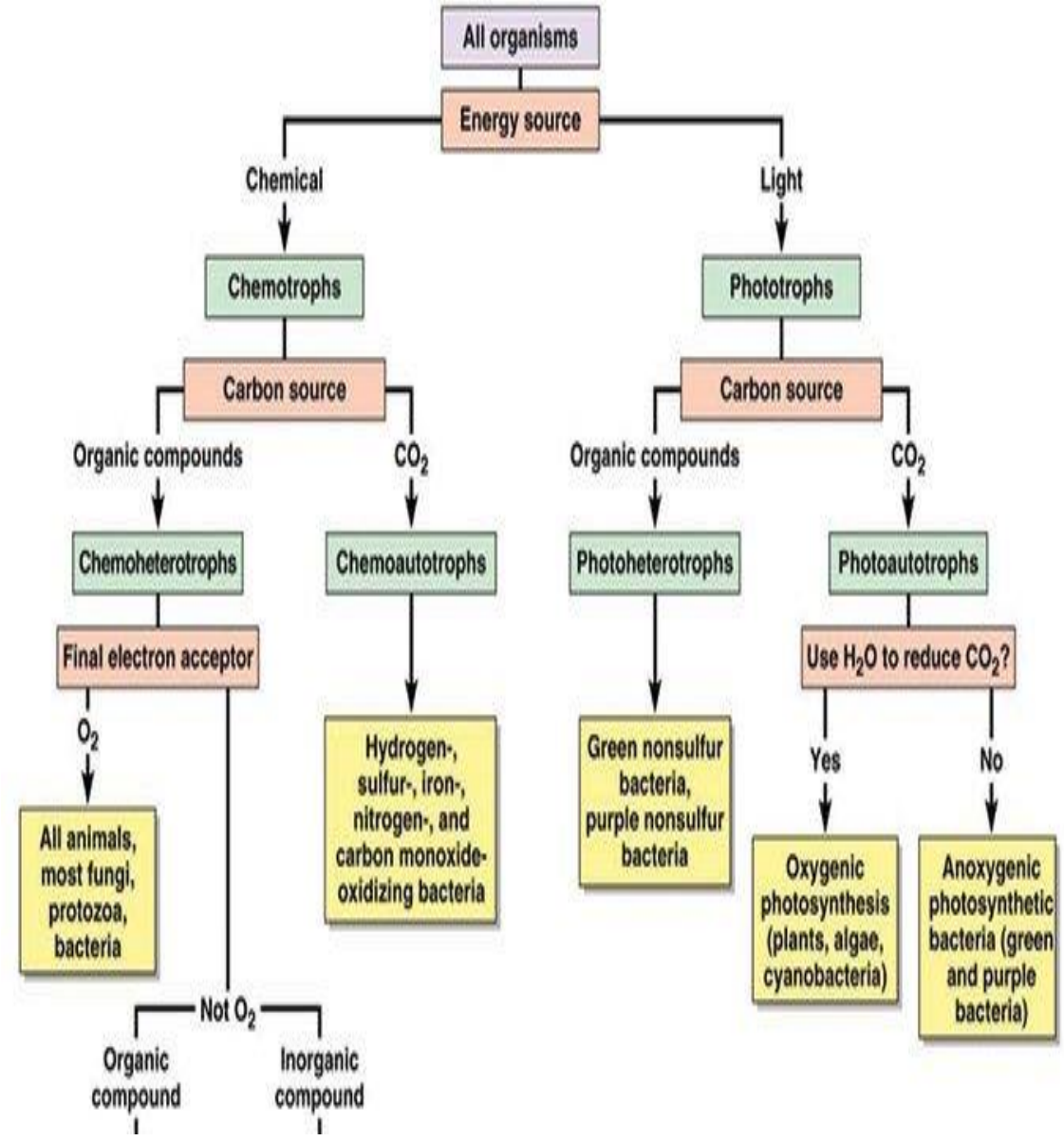
1. Physical control includes such methods of control as high or low temperature, desiccation, osmotic pressure, radiation, and filtration.
2. Chemical control refers to the use of disinfectants, antiseptics, antibiotics, and chemotherapeutic antimicrobial chemicals.
3. Sterilization is the process of destroying all living organisms and viruses.
4. Disinfection is the elimination of microorganisms, but not necessarily endospores, from inanimate objects or surfaces.
5. Decontamination is the treatment of an object or inanimate surface to make it safe to handle.
6. A disinfectant is an agents used to disinfect inanimate objects but generally to toxic to use on human tissues.
7. An antiseptic is an agent that kills or inhibits growth of microbes but is safe to use on human tissue.
8. A sanitizer is an agent that reduces microbial numbers to a safe level.
9. An antibiotic is a metabolic product produced by one microorganism that inhibits or kills other microorganisms.
10. Synthetic chemicals that can be used therapeutically.
11. An agent that is cidal in action kills microorganisms.
12. An agent that is static in action inhibits the growth of microorganisms.
13. Selective toxicity means that the chemical being used should inhibit or kill the intended pathogen without seriously harming the host.
14. A broad spectrum agent is one generally effective against a variety of Gram-positive and Gram-negative bacteria.

    A narrow spectrum agent generally works against just Gram-positives, Gram-negatives, or only a few bacteria

Questions:

1. Define the following terms: sterilization, disinfection, decontamination, disinfectant, antiseptic, and sanitizer.
2. 2. State why chemical agents are usually unreliable for sterilization.
3. . List five factors that may influence the antimicrobial action of disinfectants, antiseptics, and sanitizers.
4. 4. Describe two modes of action of disinfectants, antiseptics, and sanitizers, i.e., how they harm the microorganisms.
5. 5. Name two chemical agents that are reliable for sterilization. 6
6. . Define transient flora and resident flora and compare the two groups in terms of ease of removal.

Mind map


All organisms
Energy source
Chemical
Light
Chemotrophs
Phototrophs
Carbon source
Carbon source
Organic compounds
CO_2
Organic compounds
CO_2
Chemoheterotrophs
Chemoautotrophs
Photoheterotrophs
Photoautotrophs
Final electron acceptor
Use H_2O to reduce CO_2?
O_2
Hydrogen-, sulfur-, iron-, nitrogen-, and carbon monoxide-oxidizing bacteria
Green nonsulfur bacteria, purple nonsulfur bacteria
Yes
No
All animals, most fungi, protozoa, bacteria
Oxygenic photosynthesis (plants, algae, cyanobacteria)
Anoxygenic photosynthetic bacteria (green and purple bacteria)
Not O_2
Organic compound
Inorganic compound

UNIT- V

INUTRITIONAL TYPES OF BACTERIA

Nutrition is a substance required in biosynthesis and energy production and for microbial growth.All organisms require a Source of energy. On the basis of energy source organisms are designated as:

1. Chemotrophs: The organism that relies on chemical compounds for their energy

2. Phototrophs: The organisms which can utilize light as an energy source are known as phototrophs.

All organisms require a Source of electron for their metabolism. On the basis of electron source organisms are designated as:

1. Lithotrophs: Some organisms can use reduced organic compounds as electron donors and are termed as Lithotrophs.They can be Chemolithotrophs and Photolithotrophs

2. Organotrophs: Some organisms can use organic compounds as electron donors and are termed as organotrophs.

Some can be Chemoorganotrophs and Photoorganotrophs.

All organisms require carbon in some form for use in synthesizing cell components. All organisms require at least small amount of CO2. However, some can use CO2 as their major or even sole source of carbon; such organisms are termed as Autotrophs (Autotrophic bacteria). Others require organic compounds as their carbon source and are known as Heterotrophs (Heterotrophic bacteria).

(A) Autotrophic bacteria:

These bacteria synthesize all their food from inorganic substances (H2O, C02, H2S salts). The autotrophic bacteria are of two types:

(i) Photoautotrophs

(ii) Chemoautotrophs

I. Photoautotrophs:

These bacteria capture the energy of sunlight and transform it into the chemical energy. In this process, CO_2 is reduced to carbohydrates. The hydrogen donor is water and the process produce free oxygen. Photoautotroph has Chlorophyll pigment in the cell and its main function is to capture sunlight e.g., Cyanobacteria.

This photoautotrophic bacteria are anaerobes and have bacteriochlorophyll and bacteriovirdin pigments respectively.

Purple Sulphur Bacteria:

These bacteria have the pigment bacteriochlorophyll located on the intracytoplasmic membrane i.e., thylakoids. These bacteria obtain energy from sulfur compounds e.g., Chromatiiun. Theopedia rosea, Thiospirilium.

Green Sulphur Bacteria:

These bacteria use hydrogen sulfide (H_2S) as hydrogen donor. The reaction takes place in the presence of light and pigment termed as bacteriovirdin or bacteriopheophytin or chlorobium chlorophyll e.g., Chlorobium limicola, Chlorobacterium etc.

These bacteria take hydrogen from inorganic sources like sulphides and thiosulphates. Therefore, these bacteria are also known as photolithographs.

II.Chemoautotrophs:

These bacteria do not require light (lack the light phase but have the dark phase of photosynthesis) and pigment for their nutrition. These bacteria oxidize certain inorganic substances with the help of atmospheric oxygen. This reaction releases the energy (exothermic) which is used to drive the synthetic processes of the cell.

(a) Sulphomonas (Sulphur bacteria): These bacteria obtain energy by oxidation of elemental sulphur or H_2S, e.g., Thiobadllus, Beggiatoa.

Elemental Sulphur Oxidising Bacteria: Denitrifying sulphur bacteria oxidize elemental sulphur to sulphuric

acid e.g., Thiobacillus denitrificans

$2S + 2H_2O + 3O_2 \rightarrow 2H_2SO_4 + 126$ kcal.

Sulphide Oxidizing Bacteria: These bacteria oxidizes H_2S and release the sulphur e.g., Beggiatoa.

$2H_2S + 4O_2 \rightarrow 2H_2O + 2S + 141.8$ cal

Sulphuric acid

(b) Hydromonas (Hydrogen bacteria): These convert hydrogen into water, e.g., Bacillus pantotrophus, Hydrogenomonas.

$2H_2 + O_2 \rightarrow 2H_2O + 55$ kcal.

$4H_2 + CO_2 \rightarrow 2H_2O + CH_4 + Energy$

(c) Ferromonas (Iron bacteria): These bacteria inhabit water and obtain energy by oxidation of ferrous compounds into ferric forms. e.g., Thiobacillus ferroxidans, Ferro bacillus, Leptothrix.

$4FeCo_3 + 6H_2O + O_2 \rightarrow 4Fe(OH)_3 + 4CO_2 + 81$ kcal.

(d) Mcthanomonas (Methane bacteria): These bacteria get their energy by oxidation of methane into water and carbon dioxide.

(e) Nitrosomonas (Nitrifying bacteria): These bacteria get their energy by oxidation of ammonia and nitrogen compounds into nitrates. Nitrosomonas oxidises NH_3 to nitrites.

$NH_3 + \frac{1}{2}O_2 \rightarrow H_2O + HNO_2 + Energy$

Nitrobacter converts nitrites to nitrates.

$NO_2 + \frac{1}{2}O_2 \rightarrow NO_2 + Energy$

(f) Carbon Bacteria:

These bacteria oxidizes CO into CO_2 e.g., Bacillus oligocarbophillous, Oligotropha carboxydovorans

$2CO + O_2 \rightarrow 2CO_2 + Energy$

(B) Heterotrophic bacteria:

The heterotrophic bacteria obtain their-ready made food from organic substances, living or dead. These are of three types:

a) Photoheterotrophs

These bacteria can utilize light energy but cannot use CO_2 as their sole source of carbon. They obtain energy from organic compounds to satisfy their carbon and electron requirements. Bacteriochlorophyll pigment is found in these bacteia e.g., Purple non-sulphur bacteria (Rhodospirillum, Rhodomicrobium, Rhodopseudomonas palustris).

b) Chemoheterotrophs:

Chemoheterotrophs obtain both carbon and energy from organic compounds such as carbohydrates, lipids and proteins.

Glucose or Monosaccharide $[(CH_2O)n] + O_2 \rightarrow CO_2 + H_2O$ + Energy

There are three main categories that differ in how chemohetrotrophs obtain their organic nutrients.

(i) Saprophytic bacteria.

(ii) Parasitic bacteria.

(iii) Symbiotic bacteria.

(i) Saprophytic bacteria:

Saprophytic bacteria obtain their food from the dead and organic decaying matter such as leaves, fruits, vegetables, meat, animal feces, leather, humus etc. These bacteria secrete enzymes to digest the food and absorb it. The enzymes secreted break down the complex compounds such as carbohydrate and protein, into simpler soluble compounds, which are easily absorbed. Examples are Bacillus mycoides, B. ramosus, Acetobacter etc.

(ii) Parasitic bacteria:

These bacteria obtain their nutrition from the tissues of the hosts on which they grow. They may be harmless or may cause serious diseases. Parasitic bacteria which cause various diseases in plants and animals are known

as pathogens, e.g., Bacillus typhosus, B. anthracis, B.tetani. B.diplheriae, B.tuberculosis, B. pneumoniae, Vibrio cholerae, Pseudomonas citri etc.

(iii) Symbiotic bacteria:

Symbiotic bacteria live in close association with other organisms as symbionts. They are beneficial to the organisms. The common examples are the nitrogen-fixing bacteria, e.g., Bacillus radicicola, B. azotobacter, Rhizobium, Clostridium etc. Rhizobium spp.,B. radicicola and B. azotobacter. These bacteria live inside the roots of leguminous plants. These bacteria fix free atmospheric nitrogen into nitrogenous compounds which are utilized by the plants. In return, the plant provides nutrients and protection to the bacteria.

Photosynthesis in Bacteria

Introduction

Photosynthetic bacteria have been around for longer than the Earth's atmosphere could sustain human life. It was only recently though that scientists began to unravel the mystery of how these micro-organisms execute the mechanisms of photosynthesis.

While scientists still have not been able to put all the pieces of the photosynthetic bacteria puzzle in the right places, they are actively studying them and are gaining valuable knowledge about the way they photosynthesize and how they have evolved. In fact, they believe that these micro-organisms may have had a huge impact on why the world evolved the way it did, and may show potential for life in places deemed uninhabitable, including extreme climates like Antarctica and even other planets.

What are photosynthetic bacteria?

Much like the name suggests, these micro-organisms are special types of bacteria that contain light absorbing pigments and reaction centers which make them capable of converting light energy into chemical energy.

Cyanobacteria contain chlorophyll while other forms of bacteria contain bacteriochlorophyll. Although bacteriochlorophyll resembles chlorophyll, it absorbs light of a longer wavelength than

chlorophyll. Bacteriochlorophyll a is the most common form of bacteriochlorophyll but other forms include b, c, d, e, f and g.

Bacteria that contain bacteriochlorophyll do not use water as an electron donor and therefore do not produce oxygen. This is known as anoxygenic photosynthesis.

Cyanobacteria perform photosynthesis using water as an electron donor in a similar manner to plants. This results in the production of oxygen and is known as oxygenic photosynthesis.

Classification of Photosynthetic Bacteria

Oxygenic photosynthetic bacteria perform photosynthesis in a similar manner to plants.

They contain light-harvesting pigments, absorb carbon dioxide, and release oxygen. **Cyanobacteria or Cyanophyta** are the only form of oxygenic photosynthetic bacteria known to date. There are, however, several species of Cyanobacteria. They are often blue-green in color and are thought to have contributed to the biodiversity on Earth by helping to convert the Earth's early oxygen-deficient atmosphere to an oxygen-rich nvironment. This transformation meant that most anaerobic organisms that thrived in the absence of oxygen eventually became extinct and new organisms that were dependent on oxygen began to emerge.

Cyanobacteria are mostly found in water but can survive on land, in rocks, and even in animal shells (or fur), and in coral. They are also known to be endosymbiont, which means they can live within the cells or body of another organism in a mutually beneficial way.

Cyanobacteria also tend to live in extreme weather conditions, such as Antarctica, and are interesting to scientists because they may indicate a chance for life on other planets such as Mars.

Anoxygenic photosynthetic bacteria consume carbon dioxide but do not release oxygen. These include Green and Purple bacteria as well as Filamentous Anoxygenic Phototrophs (FAPs), Phototrophic Acidobacteria, and Phototrophic Heliobacteria. Let's look at the differences between these types of bacteria a little more closely.

Purple bacteria can be divided into two main types – the Chromatiaceae, which produce sulfur particles inside their cells, and the Ectothiorhodospiraceae, which produce sulphur particles outside their cells. They cannot photosynthesize in places that have an abundance of oxygen, so they are typically found in either stagnant water or hot sulfuric springs.

Instead of using water to photosynthesize, like plants and cyanobacteria, purple sulfur bacteria use hydrogen sulfide as their reducing agent, which is why they give off sulfur rather than oxygen. Purple bacteria are probably the most widely studied photosynthetic bacteria, being used for all sorts of scientific endeavors including theories on possible microbiological life on other planets.

Purple non-sulfur bacteria do not release sulfur because instead of using hydrogen sulfide as its reducing agent, they use hydrogen. While these bacteria can tolerate small amounts of sulfur, they tolerate much less than purple or green sulfur bacteria, and too much hydrogen sulfide is toxic to them.

Green sulfur bacteria generally do not move (non-motile), and can come in multiple shapes such as spheres, rods, and spirals. These bacteria have been found deep in the ocean near a black smoker in Mexico, where they survived off the light of a thermal vent. They have also been found underwater near Indonesia. These bacteria can survive in extreme conditions, like the other types of photosynthetic bacteria, suggesting an evolutionary potential for life in places otherwise thought uninhabitable.

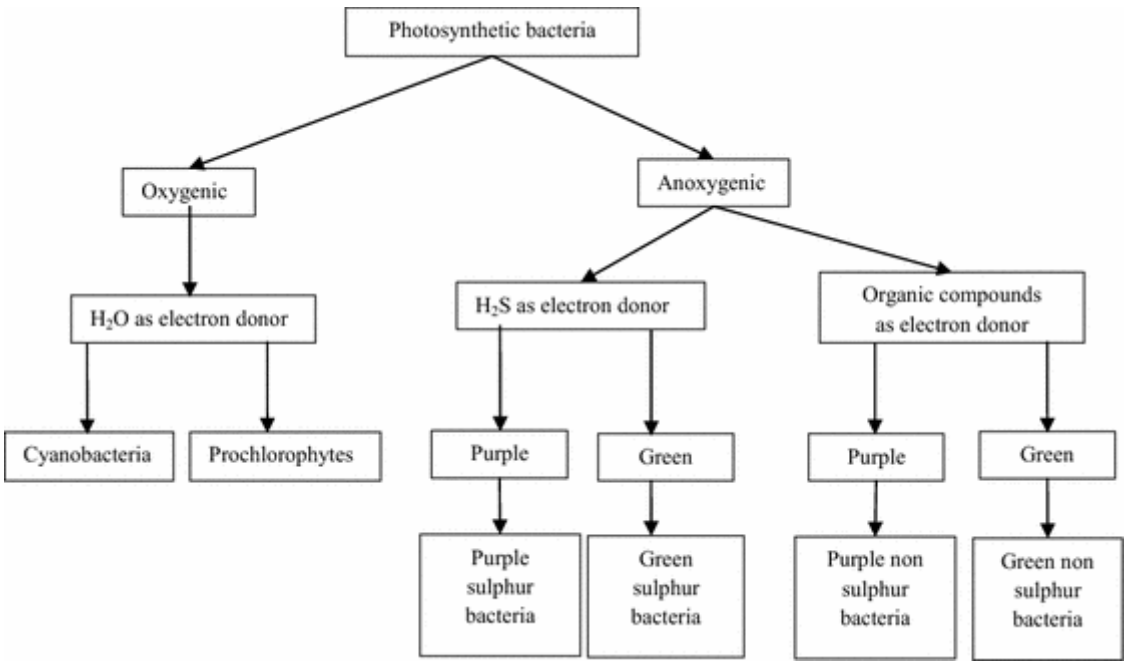
Phototrophic Acidobacteria are found in a lot of soils and are fairly diverse. Some are acidophilic meaning they thrive under very acidic conditions. However, not much is known about this grouping of bacteria, because they are fairly new, the first being found in 1991.

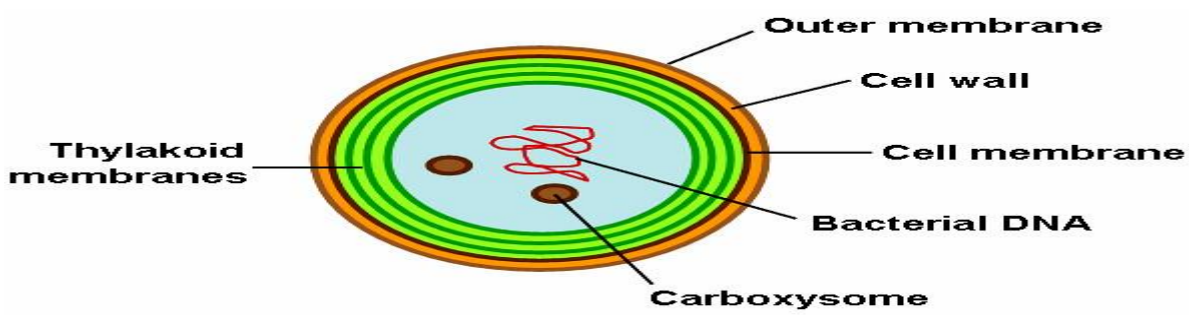
Phototrophic Heliobacteria are also found in soils, especially water-saturated fields, like rice paddies. They use a particular type of bacteriochlorophyll, labelled g, which differentiates

them from other types of photosynthetic bacteria. They are photoheterotroph, which means that they cannot use carbon dioxide as their primary source of carbon.

Green and red filamentous anoxygenic phototrophs (FAPs) were previously called green non-sulfur bacteria, until it was discovered that they could also use sulfur components to work through their

processes. This type of bacteria uses filaments to move around. The color depends on the type of bacteriochlorophyll the particular organism uses. What is also unique about this form of bacteria is that it can either be photoautotrophic, meaning they create their own energy through the sun's energy; chemoorganotropic, which requires a source of carbon; or photoheterotrophic, which, as explained above, means they don't use carbon dioxide for their carbon source.





GROWTH OF MICROORGANISMS

Microorganisms are generally found in nature (air, soil and water) as mixed populations. Even the diseased parts of plants and animals contain a great number of microorganisms, which differ markedly from the microorganisms of other environment. To study specific role played by a specific microorganisms in its environment, one must isolate the microorganisms.

The process of screening a pure Culture by separating one type of microbe from a mixture is called **isolation**.

Some common isolation methods are:

- Isolation by streak plate technique.
- Serial dilution.
- Pour plate method.
- Spread plate method.
- Exposure plate method.

ISOLATION BY STREAK PLATE TECHNIQUE

In this method the tip of a fine structure wire loop called inoculation needle consists of a wooden or glass handle with a nichrome wire, the end which is bend to form a loop, is used to transfer microbes from culture broth. The streak wires are similar to wire loop except they do not have a loop. These are used to transfer culture in colony form on solid culture medium. In such cases, the colony from solid medium is streaked on the surface of nutrient Agar medium.

In such cases, the colony from solid medium is streaked on the surface of nutrient Agar medium in a

sterile Petri dish.

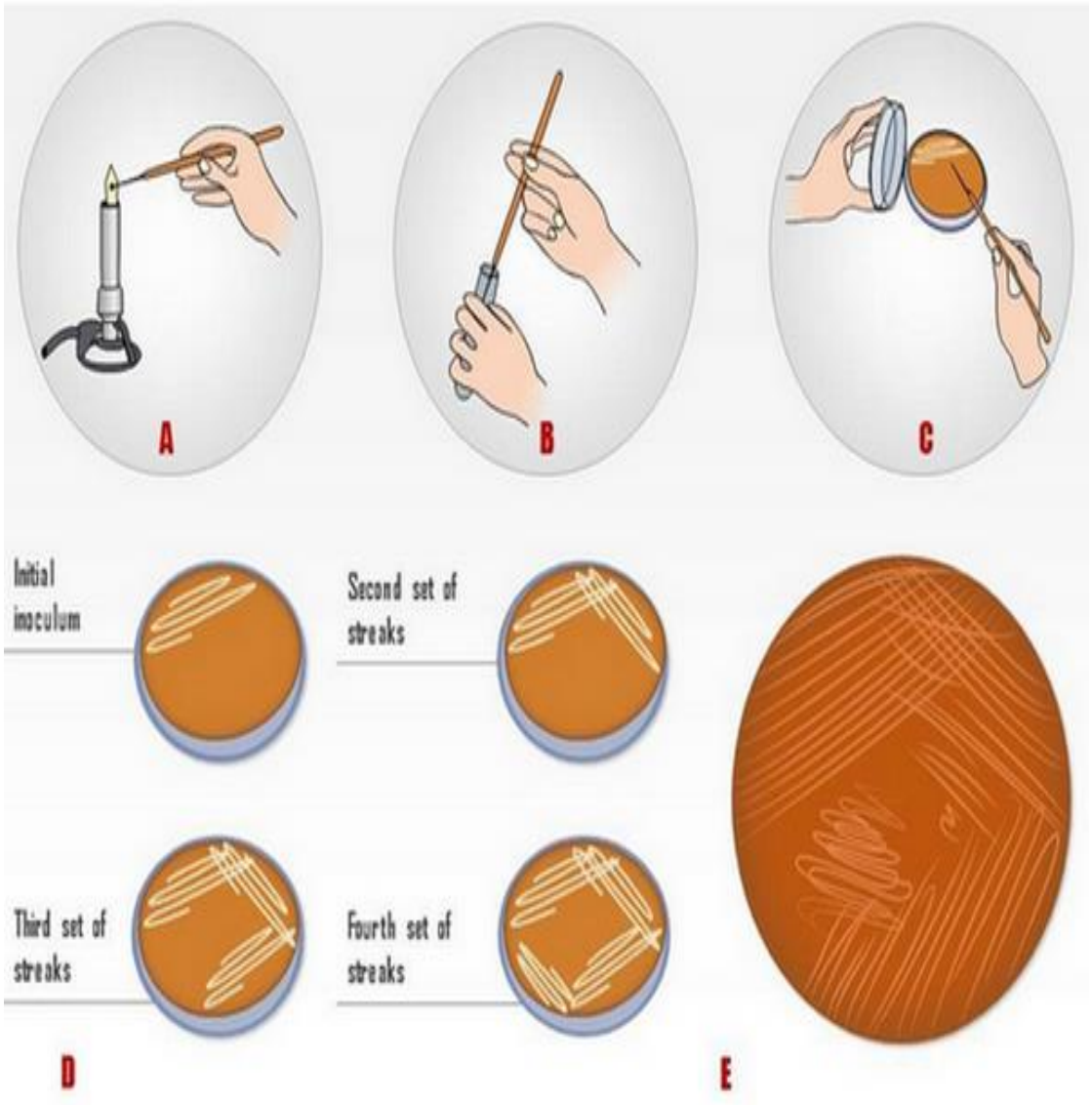
This technique consists of the following steps:

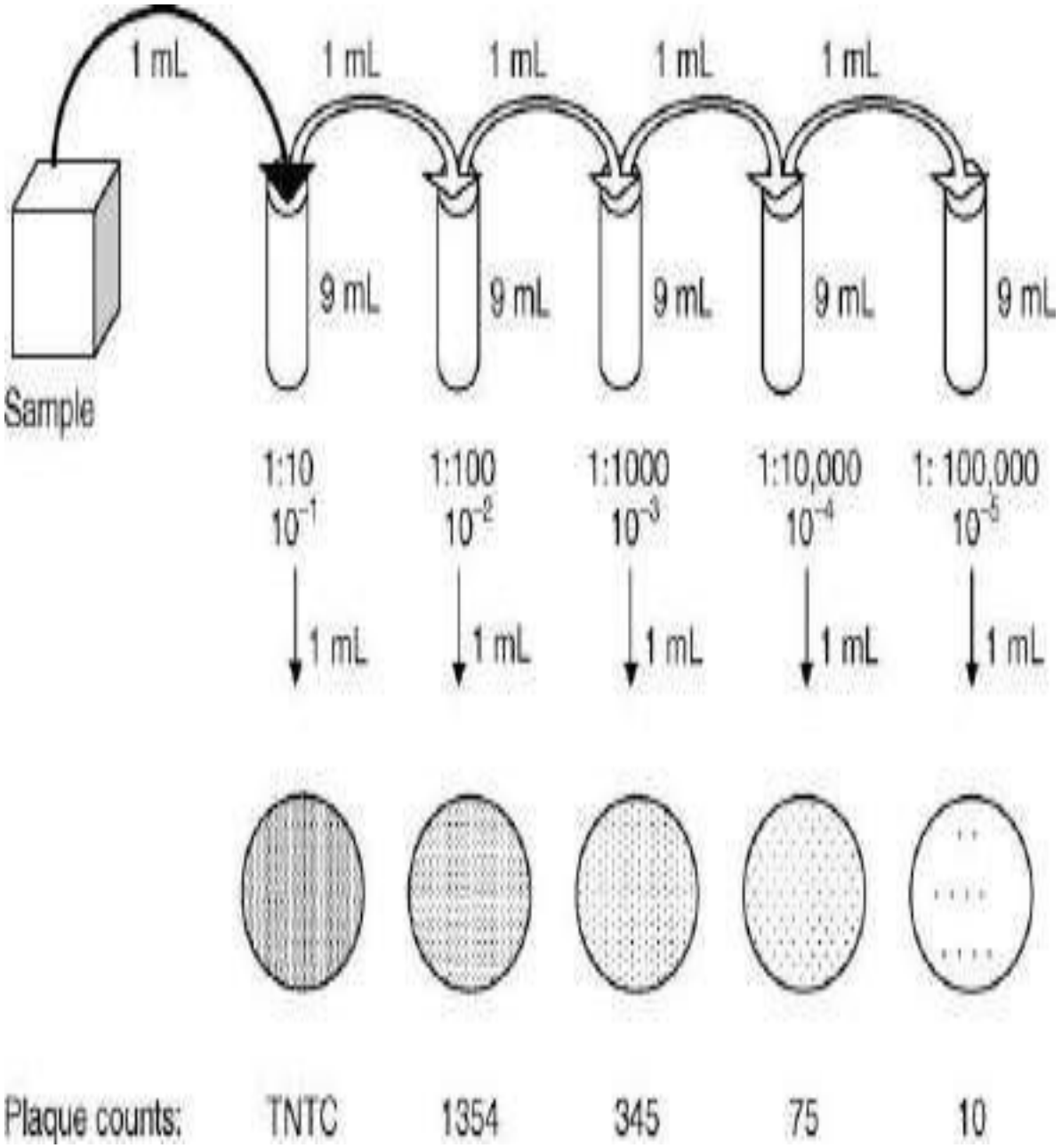
- Hold the broth culture containing tube in left hand and shake it.
- Sterilize the wire loop of the inoculation needle on burner flame.
- Remove the Cotton plug of the broth culture tube by buy a little finger of right hand.
- Flame the mouth of the test tube immediately.
- Keep the test tube in such a way as given in the figure; insert the wire loop to form a thin film and replace the Cotton plug.
- The thin film in the loop is streaked in either a zigzag manner by removing the loop backward and forward firmly. Care should be taken that loop should not be firmly pressed against the Agar surface.
- Incubate the Petri dish in incubator required temperature.
- Growth of the bacteria will be visible (after an overnight incubation) on the streaked marks.

SERIAL DILUTION

- It is a method of stepwise dilution of substances.
- The dilution factor is kept constant, resulting in a geometric progression of concentration in a logarithmic fashion.

The Streak Plate Isolation Method




1 mL
1 mL
1 mL
1 mL
1 mL
9 mL
9 mL
9 mL
9 mL
9 mL
Sample
1:10
10⁻¹
1:100
10⁻²
1:1000
10⁻³
1:10,000
10⁻⁴
1: 100,000
10⁻⁵
1 mL
1 mL
1 mL
1 mL
1 mL
Plaque counts:
TNTC
1354
345
75
10

Each dilution reduces the concentration of bacteria by a specific amount.

By calculating the total dilution over the entire series, it is possible to know how many bacteria were present when the process was started.

POUR PLATE TECHNIQUE

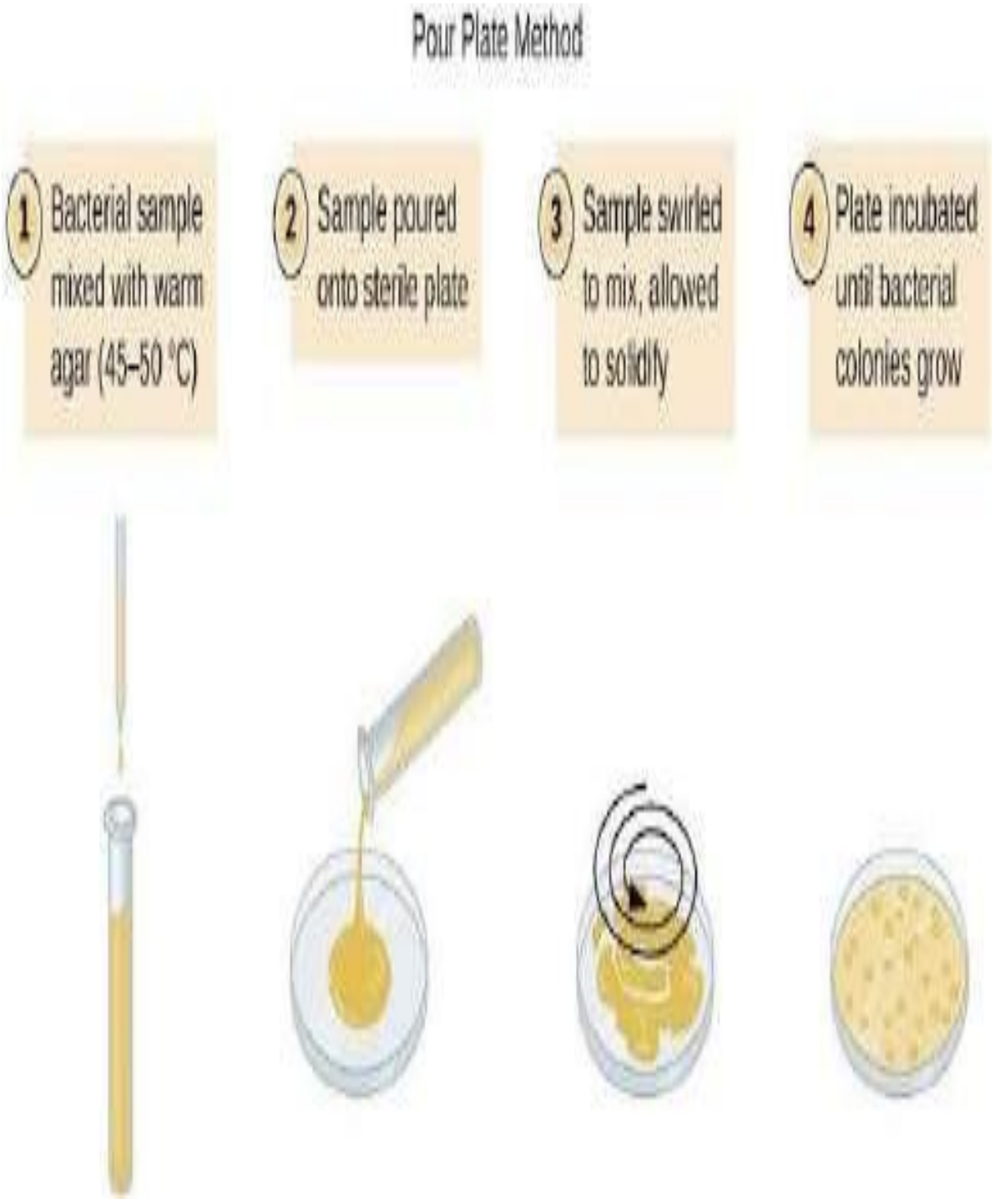
This method is used to count the number of Colony making bacteria in liquid specimen.

- In this method a fixed amount of inoculum (generally 1 ml) is taken from a broth or sample, is placed in the centre of a sterile petri plate with the help of a sterile pipette.
- Molten cooled Agar (approximately 15 ml) is then poured onto the petri plate containing inoculum and mixed well.
- After the solidification of the Agar the Petri plate is inverted and incubated at 37° Celsius for 24 to 48 hours.
- Bacteria will grow both on the surface and within media.
- Colonies which grow within medium are very small in size and maybe confluent.
- Few bacteria which grow on the Agar plate surface are of same size and appear as those on a streak plate.

Pour Plate Uses

- This method can be used to determine the number of microbes per ml in a specimen or sample.

It has the advantage of not requiring previously prepared plates and often used to assay bacterial contamination of food stuf


Pour Plate Method
1 Bacterial sample mixed with warm agar (45–50 °C)
2 Sample poured onto sterile plate
3 Sample swirled to mix, allowed to solidify
4 Plate incubated until bacterial colonies grow

SPREAD PLATE METHOD

- This is a technique used to readily quantify the amount of bacteria present in a solution.
- In this technique, the sample is diluted and then a little amount of it is added to the agar plate.
- Then the sample is spread over the agar surface evenly with the help of a spreader.
- After the colonies grow, the number of colonies is counted.

The end point of our analysis is the number of colony forming units per millilitres.

EXPOSURE PLATE METHODS

The nutrient Agar slide/ culture medium containing a plate is exposed to the atmosphere for few minutes. After intubation (overnight or more) small colonies appear on the surface of the medium which may be transferred on a fresh medium aseptically to obtain pure culture, such technique is called sub-culturing. When the transfer is from solid medium (Agar) to liquid medium (broth), the term ' picking off' is used. In such cases the colour of the colony, their size, shape, appearance, form, consistency and optical properties are recorded.

Bacterial Growth Curve

Bacterial growth

Bacterial population growth studies require cultivation of viable cells in a fresh sterile broth medium and incubation in a closed culture vessel with a single batch of medium under optimum temperature, pH, and gaseous conditions. Under these conditions, the cells will reproduce rapidly and the dynamics of the microbial growth can be charted by means of a population growth curve, which is constructed by plotting the increase in cell numbers versus time of incubation and can be used to delineate stages of the growth cycle. Growth involves an increase in cell mass and number of ribosomes, duplication of the bacterial chromosome, synthesis of new cell wall and plasma membrane, partitioning of the two chromosomes, septum formation, and cell division.

Generation time

Generation time is the time required for bacteria to grow and divide i.e. one complete cell division. Some microbes are able to divide as rapidly as once every 12 to 15 minutes, others require up to several hours, and a few very slow growing bacteria may require more than 24 hours per cell division.

Because no fresh medium is provided during incubation, nutrient concentrations decline and concentrations of wastes increase. The growth of microorganisms reproducing by binary fission can be plotted as the logarithm of the number of viable cells versus the incubation time. The resulting curve has four distinct phases

The growth of microorganism can be measured by: –

1- increase in size but this a poor criterion of growth.

2- increase in the number of bacterial cell by either counting the number of living cells (viable count) or all cells (total count).

3- measurement of some component of cell structures such as protein or DNA as an indication of microbial increase (growth) or decrease (death).

Bacterial Growth Curve

When microorganisms are grown in a suitable liquid medium (batch culture or closed system) and incubated its growth follows a definite process. If bacterial counts are carried out at intervals after inoculation and plotted in relation to time, a growth curve is obtained.

The typical growth curve is divided into the following phase:

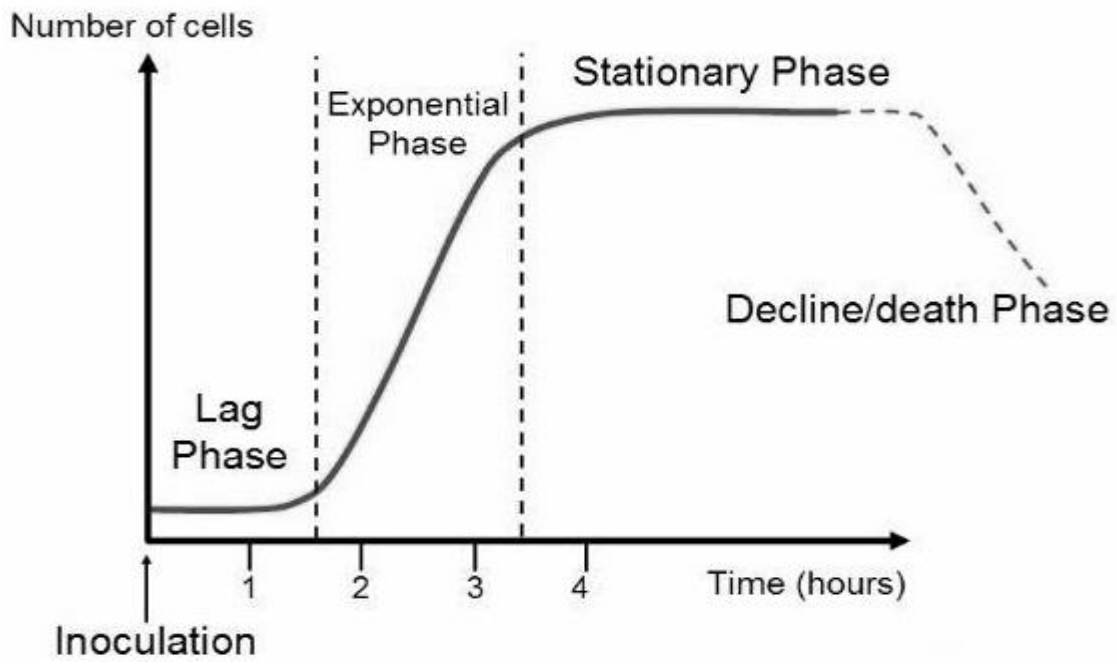
1. Lag phase
2. Log phase or exponential phase
3. Stationary phase
4. Death or decline phase

1. Lag phase

When a bacterial population is inoculated into new fresh media the cells do not reproduce immediately in a

new medium. During the lag phase, bacteria take some time adapt themselves to the new growth conditions. The lag phase is characterized by

- No cell division
- No increase in the number of cells.
- Increase in size of bacteria
- Synthesis of RNA, enzymes, and co-enzymes for physiological activities.
- Duration of the lag phase varies according to conditions and species of bacteria. For example, if the culture microorganism is taken from old culture, the duration will be longer but if the culture is fresh, duration is short. Likewise, if the culture media is different from the previous culture then duration is long because bacteria takes some more time to adapt to the fresh media.



2. Log or exponential phase

During exponential phase,

- Microorganisms start dividing at a constant rate
- Bacterial cell numbers double with time
- Rate of growth remains constant
- Bacteria have smallest size
- Generation time is shortest during this phase

• The rate of exponential growth varies between bacterial genera and is also influenced by cultural conditions.

3.Stationary phase:

A stationary phase is attained at a bacterial population level of around 109 cells per ml. During stationary phase,

there is no net increase in the number of bacterial cells

• Cell division stops due to nutrient exhaustion and accumulation of toxic products.

• The viable count remains stationary as equilibrium exists between the dying cells and the newly formed cells.

• Production of antibiotics such as Penicillin, streptomycin etc and enzymes by certain bacteria occur during this phase

• In endospore forming bacteria, sporulation occur as the bacteria enter stationary phase.

4.Phase of decline

This is the phase when the population decreased due to cell death. Since it is a closed system, there is no way to add nutrients or remove the waste products. Eventually, this leads to unfavourable conditions and a decrease in the number of living cells in the population.

Factors Affecting bacterial growth

Growth of bacteria is affected by many factors such as nutrition concentration and other environmental factors.

Some of the important factors affecting bacterial growth are:

1. Nutrition concentration
2. Temperature
3. Gaseous concentration
4. pH
5. Ions and salt concentration

6. Available water

1. Nutrient concentration:

- If culture media is rich in growth promoting substance, growth of bacteria occurs faster. Decrease in nutrient concentration decreases the growth rate.
- Different bacteria have different nutritional requirement.

The relationship between substrate concentration (nutrition) and growth rate is shown in figure.

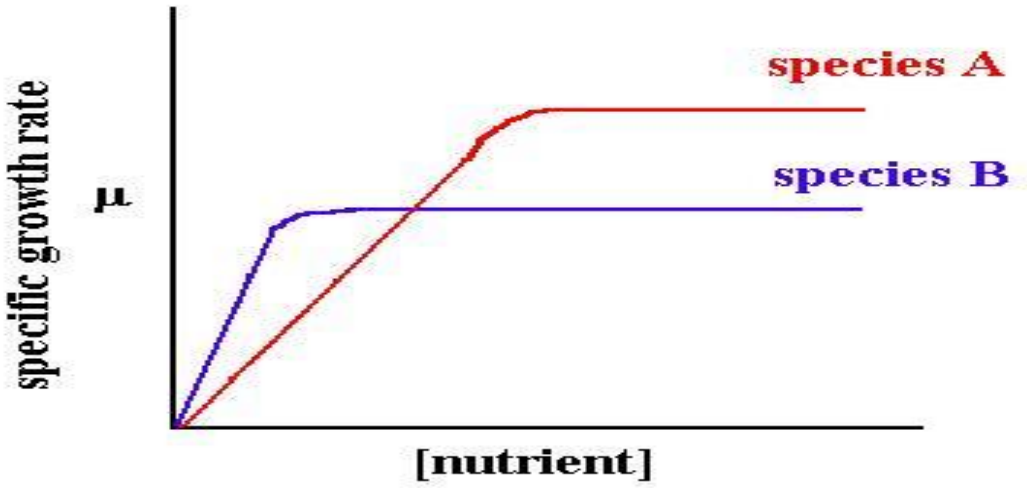


figure: nutrient vs growth rate

- With increase in concentration nutrition, growth rate of bacteria increases up to certain level and then growth rate remains constant irrespective of nutrition addition.

2. Temperature:

- Temperature affects the growth of bacteria by various ways.
- The lowest temperature that allows the growth is called minimum temperature and the highest temperature that allows growth is called maximum temperature.
- There is no growth below minimum and above maximum temperature.

- Below minimum temperature cell membrane solidifies and become stiff to transport nutrients in to the cell, hence no growth occurs.
- Above maximum temperature, cellular proteins and enzymes denatures, so the bacterial growth ceases.

The relationship between temperature and growth rate is shown in figure below.

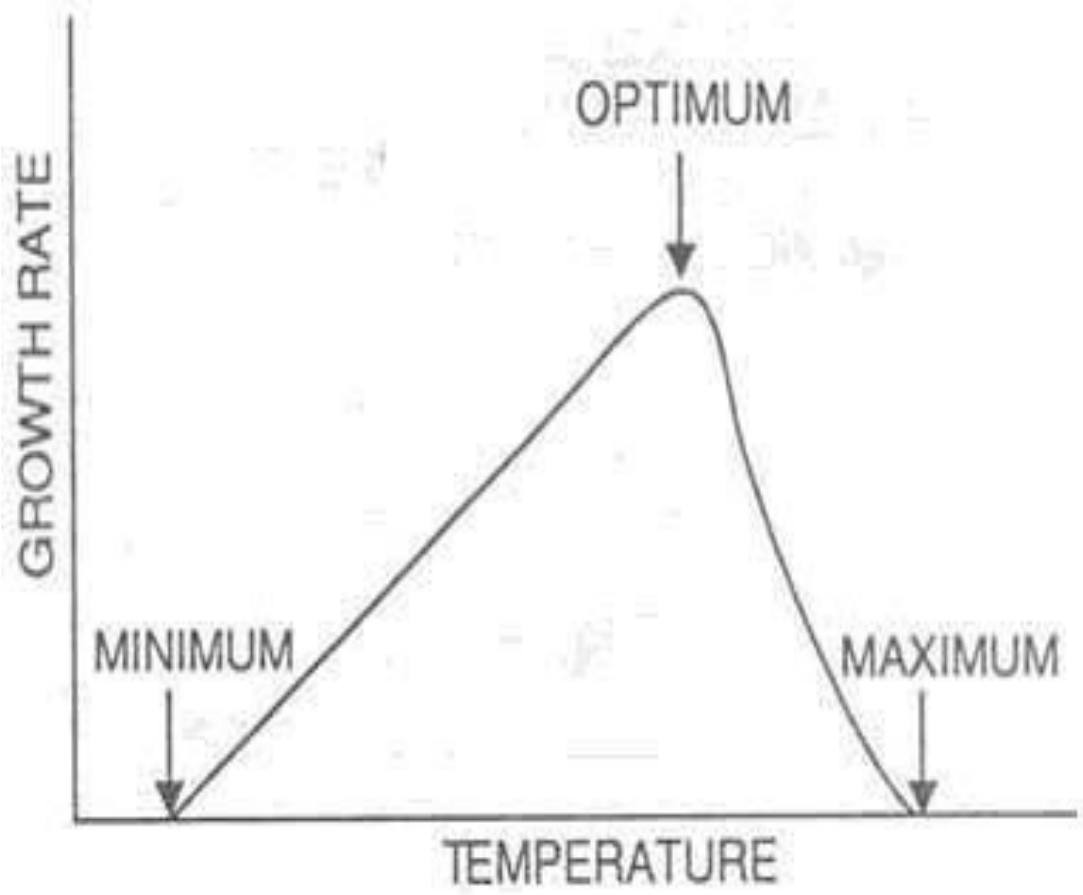


figure: temperature vs growth rate

- When temperature is increases continuously from its minimum, growth rate of bacteria increases because the rate of metabolic reaction increases with increase in temperature.
- At certain temperature the growth rate become maximum, this temperature is known as optimal temperature.
- On further increasing the temperature above optimal, growth rate decreases abruptly and completely ceases with reaching maximum temperature.

3. pH:

- pH affects the ionic properties of bacterial cell so it affects the growth of bacteria.

- Most of the bacteria grow at neutral pH (60.5-7.5). However there are certain bacteria that grow best at acidic or basic pH.
- relationship between pH and bacterial growth is given in figure below.

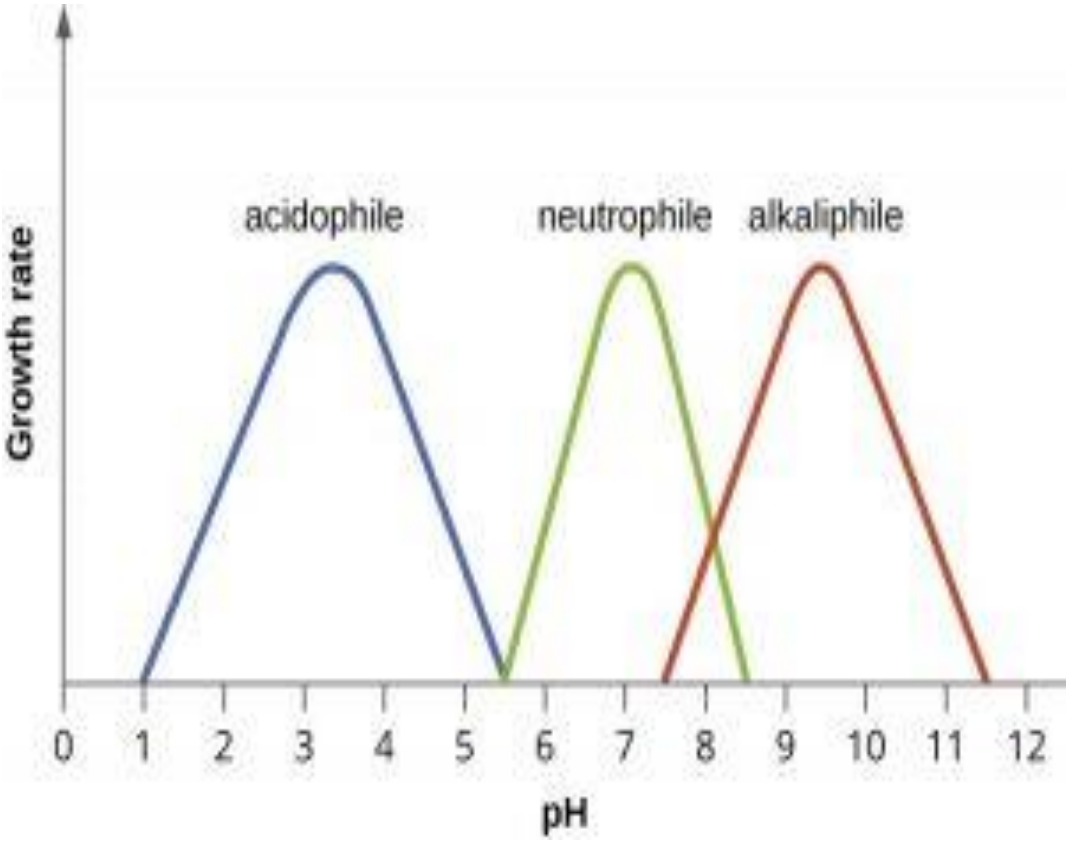


figure: pH vs growth rate

4. Ions and salt:

- All bacteria requires metal ions such as K+, Ca ++, Mg++, Fe++, Zn++, Cu++,Mn++ etc to synthesize enzymes and proteins.
- Most bacteria do not require NaCl in media however they can tolerate very low concentration of salt.
- There is some halophilic bacteria such as *Archeobacteria* that require high concentration of salt in media.

5. Gaseous requirement:

- Oxygen and carbon-dioxide are important gases that affects the growth of bacteria.
- Oxygen is required for aerobic respiration and obligate aerobic bacteria must require O2 for growth. Eg. *Mycobacterium, Bacillus*

- For obligate anaerobes Oxygen is harmful or sometime lethal. However facultative anaerobes can tolerate low concentration of O2.
- Carbon-dioxide is needed for capnophilic bacteria. Such as *Campylobacter, Helicobacter pylori*

6. Available water:

- Water is the most essential factor for bacterial growth.
- Available water in the culture media determines the rate of metabolic and physiological activities of bacteria.
- Sugar, salts and other substances are dissolved in water and are made available for bacteria

NUTRIENT TRANSPORT MECHANISMS

Nutrient uptake (i.e., solute transport) is a cellular process for acquiring molecules from the cell environment that are needed to support cell growth, metabolism and cell maintenance.

- Cells require many types of essential and non-essential nutrients.
- Cells scavenge compounds present in low to high abundance from their environments and accumulate them intracellularly.
- There are two distinct types of nutrient uptake:
- **Passive transport.** Passive transport does not require cell energy input. It occurs either by the **passive diffusion** of a molecule across the cell membrane, or by the **facilitated diffusion** of the molecule aided by a specialized membrane protein.
- **Active transport.** Active transport of a nutrient requires a dedicated solute transport system and input of cell energy. There are several types of systems that are differentiated by the mechanism of molecule uptake, the energy source, and the types of proteins present. Examples include the **ABC-type transporters**, **symporters, antiporters, and group translocation transporters**.
- Solute transport systems are also used to maintain intracellular ion levels, and to export cell waste materials and toxins.

GENERAL BACKGROUND ON NUTRIENT UPTAKE

E. coli, like most microorganisms, thrives in environments that are often limiting for the many types of nutrients needed to support cell growth. To accumulate these molecules the cell employs dedicated nutrient uptake

systems called solute transporters. Most of these require energy input in form of the proton motive force or ATP hydrolysis to drive nutrient uptake into the cell.

To accomplish nutrient uptake, the cell must overcome three major obstacles.

The number and types of nutrients used by the cell is large. Some nutrients are essential and the cell cannot survive without them.

Many other nutrients are not essential for *E. coli* cell survival but they are acquired by the cell in order to conserve energy...

NUTRIENT UPTAKE

I. PASSIVE TRANSPORT ACROSS MEMBRANES

Entry of solutes into the cell by simple diffusion is generally limited to a few types of molecules since the cytoplasmic membrane forms a hydrophobic barrier to most types of nutrients. The driving force for simple diffusion depends on the solute concentration gradient across the membrane. The molecules will flow from the region of high concentration to the region of lower concentration. Thus, the concentration inside the cell can never be higher than the level outside the membrane.

There are two types of passive transport systems called passive diffusion and facilitated diffusion. They differ in that one type employs a special protein to assist in moving the molecule across the membrane while the other does not.

Facilitated vs Passive Diffusion

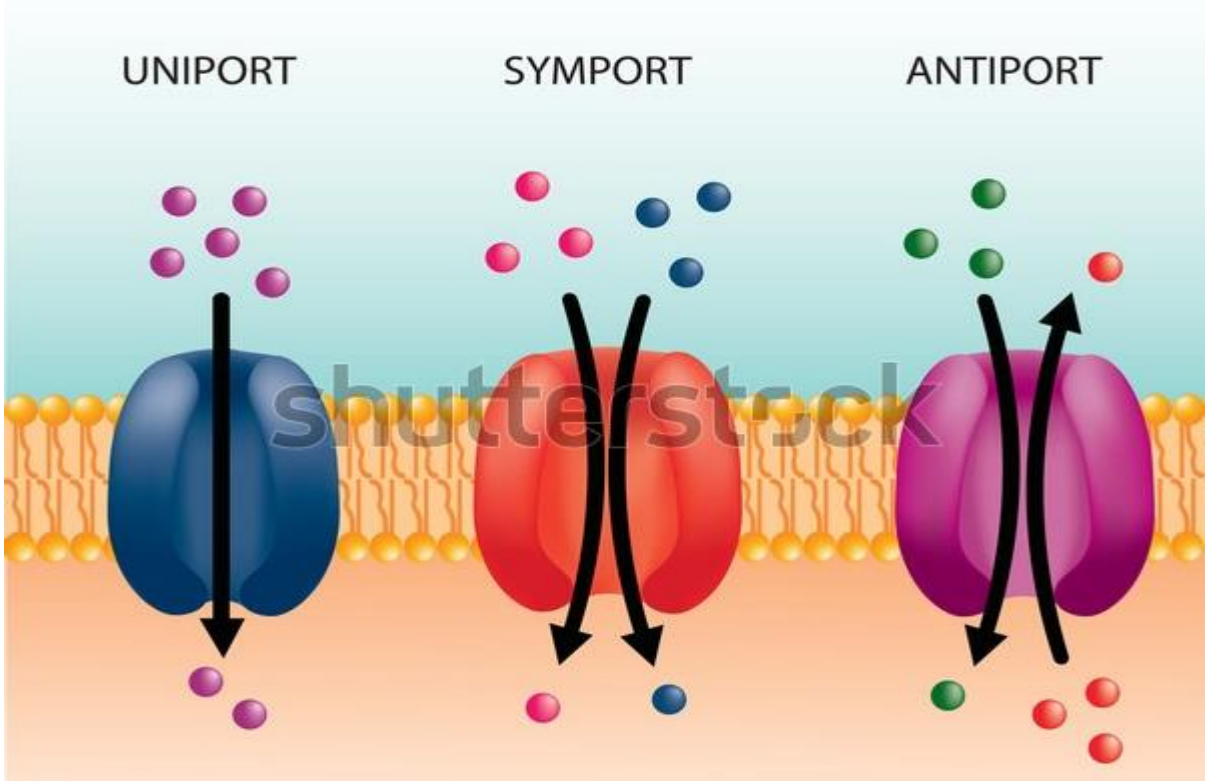
The solute molecule shown above (ammonia) enters the cell at a much faster rate via the ammonia-specific facilitator protein compared to passive diffusion.

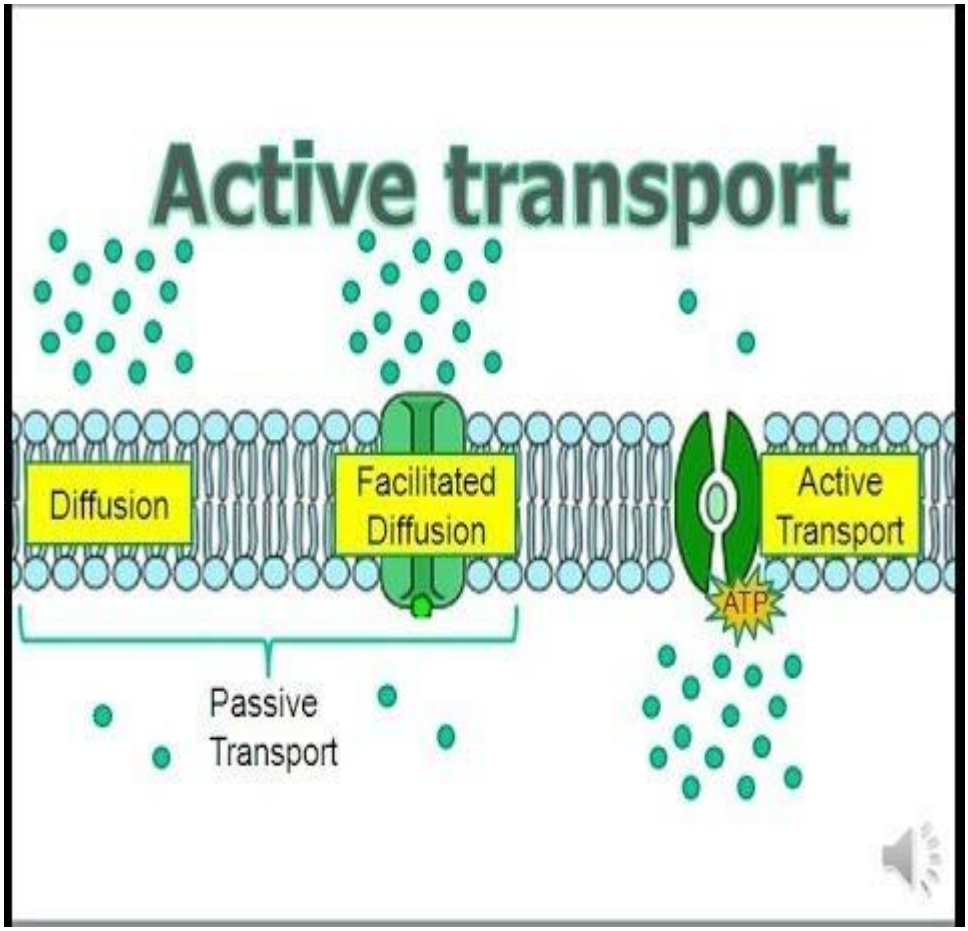
1. **Passive diffusion.**

- Passive diffusion of a nutrient across the membrane does not require cell energy or any cell proteins. Several types of gasses (e.g. O_2, N_2, CO_2) plus small, uncharged polar molecules (e.g. H_2O, glycerol, urea) can pass directly through the cytoplasmic membrane at reasonable rates as they are neither particularly hydrophobic nor hydrophilic in nature. The rate of diffusion of most types of molecules is generally very slow.
- The direction of molecule movement across the membrane is determined entirely by the concentration of the molecule on the outside versus the inside of the cell. When the concentration is higher outside, molecules will

flow into the cell. Conversely, if the concentration is higher inside, molecules flow out of the cell. In reality, passive diffusion across the cytoplasmic membrane does not generally supply sufficient amounts of required nutrients to support cell growth.

2.Facilitated diffusion. This process is similar to passive diffusion except that the cell employs a specialized protein embedded in the membrane that "facilitates" the movement of the molecule across it. This allows entry of charged and polar molecules that would otherwise be excluded by the hydrophobic membrane barrier. Without a facilitator protein, highly abundant molecules in the environment could not otherwise cross the membrane.





There are two types of facilitator proteins called carrier proteins and porin proteins. The former are always located in the cytoplasmic membrane and the latter are always located in the outer membrane. Both proteins increase the rate of molecule diffusion across the membranes but they work in different ways.

- **Carrier proteins (uniporters)**

  Operation of a Uniport System

  The potassium ion transporter is an example of a uniport system. This animation demonstrates directional movement of K^+ along a gradient and substrate specificity.

- **Porin proteins**

II. ACTIVE TRANSPORT ACROSS THE CYTOPLASMIC MEMBRANE

The majority of the nutrients required for cell metabolism are taken up by active transport systems. This process is carried out by one or more proteins located in the cytoplasmic membrane or associated with it. All of these systems require the expenditure of cell energy supplied in the form of ATP, the proton motive force, or for some sugar transporters, by the high energy compound, phosphoenylpyruvate (PEP). Active transport systems are highly specific for an individual molecule or class of structurally related compounds.

Since active transport is driven by the expenditure of cell energy, it allows the cell to accumulate molecules to a much higher concentration inside the cell (i.e., the cytoplasm) relative to the outside of the cell (i.e., the periplasm, or cell exterior). The synthesis and/or activity of many of these active transport systems are often regulated depending on the cellular needs.

Types of Active Transport Systems

Active transport systems are divided into three general classes that are named according to their component parts or their mechanism of action. In most cases the solute is transported from the periplasm to the cell interior (i.e., cytoplasm) in an unmodified form. In a few cases the solute is chemically altered during its transfer across the cytoplasmic membrane (e.g., group translocation).

1. **ABC transporters.**
   - In Gram-negative bacteria,
   - The Gram-positive bacteria and the archaea
   - Iron III transport in Gram negative bacteria.
   - Acquisition of Fe^{3+} ions is a more complicated process than for uptake of most nutrients

Operation of ABC-type Transport System

This ABC-type transport system imports solute molecules into the cell. The solute molecule shown in green is bound by the blue periplasmic binding protein which passes the solute to the cognate membrane spanning protein. The energy released by the hydrolysis of ATP to ADP and Pi (inorganic phosphate) drives the solute molecule into the cytoplasm.

2. **P-type ATPases.**
3. **Secondary transport systems.**

The two types of secondary transport systems are defined by the directions of solute and ion movement (i.e., co-transported or antiported).

- **Symporters**
- **Antiporters**

Operation of a Symport System

The sugar disaccharide (green molecule) transporter is an example of a symport system. Sugar uptake is driven by a proton entering the cell (i.e., by a proton gradient or the pmf).

Operation of an Antiporter System

The Na+/H+ antiport system exchanges Na+ for H+. Sodium export is driven by the proton gradient (pmf).

4. Group translocation systems.

- The phosphotransferase system (PTS).
- *E. coli* fatty acid transport.

Operation of a PTS-type Transport System

The Glucose-specific PTS transporter is an example of a group translocation system. Here, glucose (the green hexagon) located in the cell periplasm space is bound by the membrane protein Enzyme IIC. Energy from PEP is used to drive glucose uptake via a phosphorylation cascade where glucose is ultimately phosphorylated as it enters the cell.

Summary:

- Nutrients enter the cell via passive or active transport mechanisms.
- Passive transport mechanisms do not require energy input and involve simple diffusion of solutes across the membrane.
- Passive transport can occur with or without involvement of a protein to facilitate diffusion.
- Active transporters always require energy input and involve dedicated transport proteins embedded in the cytoplasmic membrane.
- The three general types of active transporters include the ABC transporters, the secondary transporters and the group translocation systems.
- ABC transporters employ a periplasmic solute binding protein, a membrane intrinsic transport protein and an ATP hydrolyzing protein to drive solute uptake.
- Secondary transporters import solutes either by co-transport (symport) or by molecule exchange (antiport of an ion). The driving force for most secondary transporters is the proton motive force; alternatively, the gradient of the co- or anti- transported ion drives transport.
- Group translocation systems modify the solute upon cell entry. PEP or ATP provide the energy for the modification reaction.
- Molecules are accumulated against an increasing concentration gradient during active transport.

Questions

1. What are the different terms associated with microbial nutritional types? How can these terms be combined to define the nutritional types of microbes in terms of their sources of carbon, electrons, and energy?
2. What are macroelements and why are they important to a cell? What are growth factors and what is their significance to a cell?
3. What is the importance of nutrient uptake for a cell? What are the common features of nutrient uptake by bacteria?
4. What is transported into a bacteria cell by passive diffusion and how does this affect a bacterial cell?
5. Explain diffusion (passive and facilitated) and active transport.
6. What are the 3 types of active transport? Be able to diagram each processes. What is required for each of these processes? How are they similar, how are they different?
7. Why is iron uptake important for a cell? What is used to accomplish this?

Basic terms

Culture- population of microorganisms grown under well-defined condition.

Pure culture- a culture containing only one species of microbe.

Mixed culture-when a particular species of microbe is present in a very small number in comparison to the total number of microorganisms, such culture is called mixed culture.

Species- a collection of bacterial cells which share an overall similar pattern of traits in contrast to other bacteria whose pattern differs significantly.

. Sterilization

Sterilization is the process of destroying all living organisms and viruses. A sterile object is one free of all life forms, including bacterial endospores, as well as viruses.

2. Disinfection

Disinfection is the elimination of microorganisms, but not necessarily endospores, from inanimate objects or surfaces.

3. Decontamination

Decontamination is the treatment of an object or inanimate surface to make it safe to handle.

4. Disinfectant

A disinfectant is an agents used to disinfect inanimate objects but generally to toxic to use on human tissues.

5. Antiseptic

An antiseptic is an agent that kills or inhibits growth of microbes but is safe to use on human tissue.

6. Sanitizer

A sanitizer is an agent that reduces microbial numbers to a safe level.

7. Antibiotic

An antibiotic is a metabolic product produced by one microorganism that inhibits or kills other microorganisms.

8. Chemotherapeutic synthetic drugs

Synthetic chemicals that can be used therapeutically.

9. Cidal

An agent that is cidal in action will kill microorganisms and viruses.

10. Static

An agent that is static in action will inhibit the growth of microorganisms.

Strain- is strain is a subset of a bacterial species differing from other bacteria of the same species by some minor but identifiable difference.

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