STUDY MATERIAL FOR B.SC MICROBIOLOGY

FUNDAMENTALS OF MICROBIOLOGY AND MICROBIAL DIVERSITY

SEMESTER – I

ACADEMIC YEAR 2023-24

INDEX

UNIT – I

INTRODUCTION TO MICROBIOLOGY

Microbiology is the study of living organisms of microscopic size, which include bacteria, fungi, algae and protozoa and the infectious agents at the borderline of life that are called viruses. It is concerned with their form, structure, reproduction, physiology, metabolism and classification. It includes the study of their distribution in nature, their relationship to each other and other living organisms, their effects on human beings and on other animals and plants, their abilities to make physical and chemical changes in our environment and their reactions to physical and chemical agents.

Most **microorganisms** are unicellular in which all the life processes are performed by a single cell. All living cells contain protoplasm which is a colloidal organic complex consisting of largely proteins, lipids and nucleic acids.

HISTORY OF MICROBIOLOGY

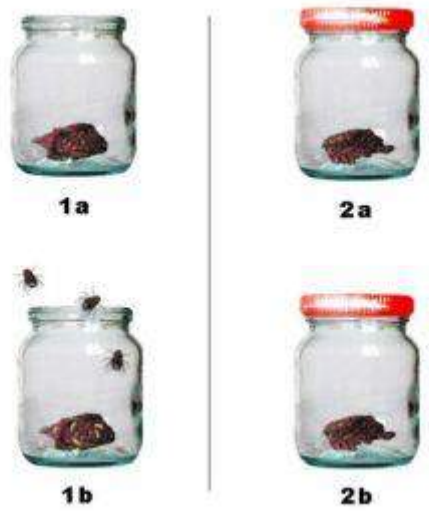
The existence of microbial world was unknown until the inventions of microscopes, which were invented at the beginning of 17th century. The discoverer of the microbial world was a Dutch merchant **Antony van Leeuwenhoek** (1632-1723) with his microscope. His microscopes were able to give clear images at magnifications from about 50 to 300 diameters. Leeuwenhoek's place in the scientific history depends on the range and skill of his microscopic observations. He studied almost every conceivable object that could be looked through a microscope. He described the microbial world he observed as 'animacules' or 'little animals. All the main kinds of unicellular organisms that we know today – protozoa, fungi, algae, & bacteria were first described by Leeuwenhoek. He was first to describe the Spermatozoa, RBC, free living as well as parasitic protozoa & the bacteria which he called animalcules (small animals). He communicated all his observations to the British Royal Society in a series of letters. Leeuwenhoek's discovery of the animalcules & other microbes revealed the presence of a hitherto unknown world – the microbial world.

'Microbiology' has been delayed till late 19th century. The principal reasons for this long delay seems to have been technical ones. After Leeuwenhoek's discovery of microorganisms, the origin of microbes became the subject of discussion. Some Scientists believed that animalcules were formed spontaneously from non-living materials, whereas others (including Leeuwenhoek) believed that they were from seeds or germs of these animalcules, which were always present in the air.

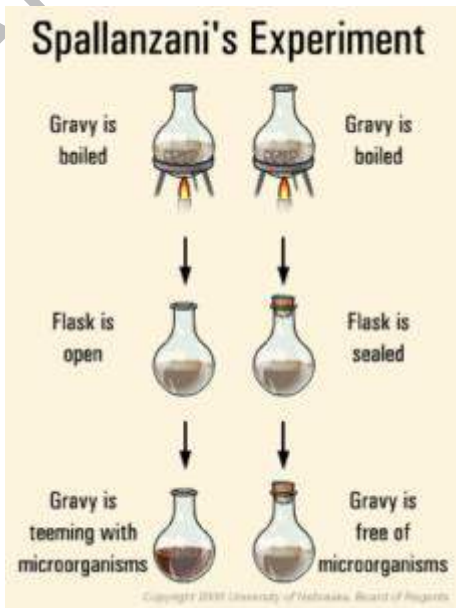
Spontaneous Generation

The belief in the spontaneous formation of living beings from non-living matter is known as Doctrine of spontaneous generation (SG). This controversy existed for a long time. It became difficult to disprove this doctrine, because of lack of experimental proof.

Later **Francesco Redi** in 1665 performed experiments and showed that maggots that develop in putrefying meat are the larval stages of flies and will never develop in putrefying meat if it is protected from flies laying eggs. He was the first to disprove SG of animals.



Lazzaro Spallanzani (1729-99) was the first to provide evidence that microorganisms do not develop spontaneously. He boiled beef broth for an hour and then sealed the flasks. No microbes appeared following incubation.



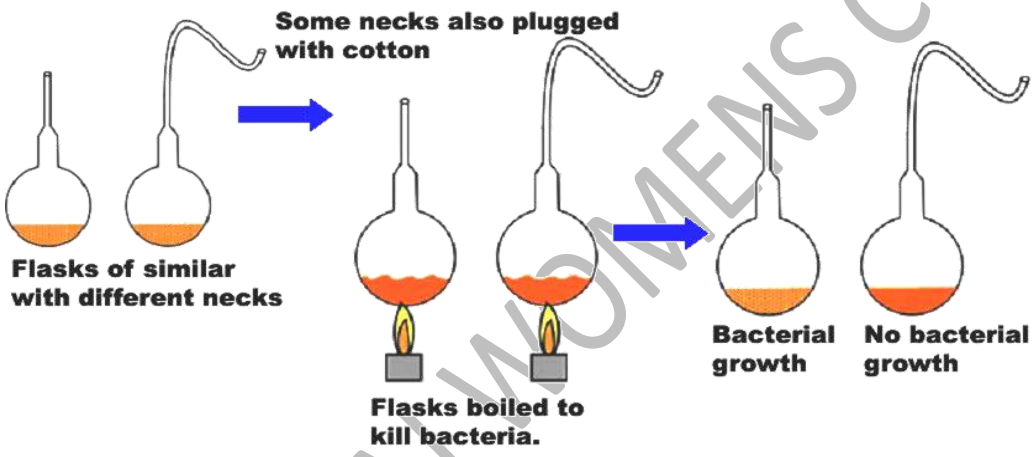
John Needham (1713-81) insisted that air was essential for SG of microbes. By sealing the flasks, the air had been excluded. This argument was answered after 60 – 70 years independently by two other scientists.

Franz Schule (1815-73) passed air through strong acid solutions into boiled infusions.

H.Schroder and T.Von Dusch (About 1850) passed air through cotton into flasks containing heated broth. Thus the microbes were filtered out of the cotton fibres and no microbial growth. Basic technique of plugging bacterial culture tubes with cotton stoppers was initiated.

Archimede Pouche (1859) published an extensive report favouring SG.

Louis Pasteur (1822-1895) the immortal French scientist, performed various experiments to disprove S.G. He developed a flask with a long, narrow gooseneck opening through which untreated and unfiltered air could pass in or out, but the germs settled in gooseneck. As germ free air entered the flask no microbes appeared in the infusion.



In 1862, Louis Pasteur conducted experiments to disprove the theory of Spontaneous Generation. He prepared flasks, with long, narrow, goose-neck openings heated the nutrient broth in the flask and thus the air carrying the germs were allowed to settle in the goose-neck. When the flasks were cooled, the air entering through the gooseneck retained the germs, and under these conditions the broth remained clear. He also showed by further studies that 'used' cotton filters, when examined under the microscope, revealed the presence of microscopic organisms.

John Tyndall (1820-1923) proved that dust carried the germs. He showed that sterile infusions placed in a dust free chamber could remain sterile indefinitely even if kept exposed to air. During his experimentation he concluded that bacteria have phases one relatively. thermolabile (growing phase destroyed by boiling for 5 min.) and one thermo resistant (bacterial spores cannot be destroyed even by boiling for 5 ½ hours). He developed a method of sterilization by discontinuous heating, later called Tyndallization, which could be used to kill all bacteria in infusions. Allowed the infusion to stand for a certain period, before applying heat to permit the

germination of spores with a consequent loss of their heat resistance. Then boiled to kill bacteria. He found that discontinuous boiling for 1 min on 5 successive occasions would make the infusion sterile whereas continuous boiling for 1 hr. would not. Pasteur and Tyndall's experiments finally disproved the Doctrine of Spontaneous generation (S.G.).

ROLE OF MICROBES IN FERMENTATION

Cagniard Latour; Theodor Schwann; F. Kutzing independently showed that microbes are involved in fermentation of sugar to alcohol.

Louis Pasteur continued his work and found that fermentation of fruits and grains, resulting in alcohol was brought out by microbes. Pasteur suggested that good quality fermented products can be obtained by selecting proper microbe. The other unfavourable microbes can be avoided by heating the fruit juice at 62.8 C for 30 min.

This process is called Pasteurization and is widely used in fermentation industries. This short heating process kills pathogenic and spoilage microorganisms but does not sterilize the liquids completely.

During his studies on the butyric fermentation, Pasteur discovered the existence of forms of life, which can live only in the absence of free oxygen. He introduced the terms aerobic and anaerobic to designate life in the presence and absence of oxygen respectively. Pasteur described that fermentation is life without air. Some strictly anaerobic microorganisms such as the butyric acid bacteria are dependent on fermentative mechanisms to obtain energy.

Most of the organisms require oxygen to oxidize organic compounds to CO2. Such oxygen linked biological oxidation known as aerobic respiration provides energy that is required for maintenance and growth.

Facultative anaerobes: Many other microorganisms including certain yeasts are facultative anaerobes which have two alternative pathways of energy yielding mechanisms – in the presence of oxygen they employ aerobic respiration – in the absence of oxygen, they employ fermentation.

$$\text{Ex: Sugar} \xrightarrow[\text{No } O_2]{\text{yeast}} \text{Alcohol} + CO_2$$

$$\text{Sugar} \xrightarrow[O_2]{\text{yeast}} CO_2, \text{ no Alcohol}$$

The above process was demonstrated by Pasteur. Fermentation is a less efficient energy yielding process than aerobic respiration, because the part of the energy present in the substance degraded is still present in the organic end products.

At the same time **Ferdinand Cohn** demonstrated that certain bacteria could produce spores, which are heat resistant.

GERM THEORY OF DISEASES

Von Plenciz (1762) described that living agents are the cause of disease. Different germs are responsible for different diseases in 1836

A. Bassi recognized that the fungus was the causative organism for disease in silkworm.

In 1845 **M J Berkeley** had proved that Potato Blight of Ireland was caused by fungus.

J L Schonlein showed that certain skin diseases of man are caused by fungal infections.

During this period **Pasteur** worked on silkworm disease and isolated the parasite, causing disease. Pebrine is caused by a protozoan rather than by a bacterium. He also showed that silkworm farmers could eliminate the disease by using only healthy and disease-free caterpillars for breeding stock. He also worked on anthrax, a disease of cattle and sheep. He isolated the microbes from diseased animals.

Robert Koch (1876) concluded the germ theory of disease by working on anthrax disease on animals (sheep). His experiments and observations led to the establishment of Koch's postulates, which provided the guidelines to identify the causative agent of infectious diseases.

He discovered the typical bacilli with squarish ends in the blood of cattle that had died of anthrax. He grew these bacteria in cultures in his laboratory, examined them microscopically to be sure he had only one kind present, & then infected them into other animals to see if these became infected & develop clinical symptoms of Anthrax.

From these experimentally infected animals he isolated microbes like those he had originally seen that died of Anthrax this was the first time a bacterium had been proved to be the cause of an animal disease.

This series of observations led to the establishment of Koch's postulates – which provided guidelines to identify the causative agent of an infectious disease.

Koch's Postulates:

a). The microorganism must be present in every case of the disease.

b). The microorganism must be isolated from the diseased host and grown in pure culture.

c). The specific disease must be reproduced when a pure culture of microorganism is inoculated into a healthy susceptible host.

d). The microorganism must be recoverable once again from the experimentally infected host.

He also demonstrated the biological specificity of disease agents. These developments led to the creation of institutions both in Paris for Pasteur and in Berlin for Koch. Pasteur's group concentrated on how the recovery and immunity are brought about in diseased animal whereas Koch's group conducted experiments on the etiology of major infectious diseases.

PURE CULTURE METHODS

O. Brefled introduced the practice of isolating single cells of fungi and their cultivation on solid media, which were obtained by adding gelatin to the liquid medium. His methods were not suitable for bacteria. Pure cultures of bacteria were 1st obtained by **Joseph Lister** by serial dilution in liquid media. Bacterium lactis was isolated into pure culture in this method.

In 1864 **Joseph Lister** developed antiseptic method of surgery. **Koch** developed pure culture technique for bacteria. He developed streak plate method and Pour plate method for this purpose. At first, Koch experimented with the use of sterile cut surfaces of potatoes which he placed in sterile covered glass dishes & then inoculated with the bacteria. The cut surfaces of potatoes had disadvantages firstly moist surface allowed motile bacteria to spread, secondly the substrate was opaque & examination of bacterial colonies is difficult.

Later Koch thought that if a liquid medium could be solidified by the addition of a solidifying agent such as gelatin. Koch Poured the molten gelatin medium on glass plates & allowed to solidify. After solidification the surface was streaked with a cotton swab dipped into bacterial suspension & then Growth was found along the streak & single colonies were found at the end of the streak.

Although gelatin was useful as a solid base in media, it had certain disadvantages.

1. It is a protein that is susceptible to microbial digestion

2. A change from gel to a liquid occurs at a temperature of about 280C while for most bacteria an incubation temperature of 30-370C is optimum

With the help of his colleague **Dr. Hesse**, he replaced the solidifying agent gelatin (Protein) with agar (a complex polysaccharide obtained from red algae) which melts at 98o C and solidifies at 44o C. Agar is a complex polysaccharide extracted from the red algae is not easily degraded by most bacteria. For these reasons agar quickly replaced gelatin as a hardening agent. Koch developed nutrient broth & its solid counterpart nutrient agar. Koch also developed the nutrient medium for the growth of bacteria.

BEIJERINCK and WINOGRADSKY

- They developed the technique of 'Enrichment culture"

- By modifying the composition of the medium or incubation conditions it is possible to isolate or specific organisms from a mixed population.

- **Francois Appert** found that highly perishable foodstuff can be preserved for long time by enclosing them in airtight containers and heating the containers. This process is known as Appertization and is the principle of food canning.

SCHRODER AND VON DUSCH passed air through cotton into flasks containing heated broth. Thus the microbes were filtered out from the air by the cotton fibres so that growth did not occur & a basic technique of plugging bacterial culture tubes was initiated.

JOHN TYNDALL concluded that some microorganism exist in two forms that is – a heat labile form (Vegetative) & heat resistant form (endospores). He developed a method of sterilization by discontinuous heating, called **Tyndallisation** which could be used to kill all bacteria in infusions.

PROTECTION AGAINST INFECTION:

Edward Jenner in 1798 used Cowpox Virus to immunize people from small pox. But the science behind this technique was not known at that time.

Frederick Loeffler (1884) discovered Diphtheria bacillus and demonstrated the production of toxin in lab flasks.

Behring and Shibasaburo Kitasato devised a method of immunity by introducing their toxins into animals so that an antitoxin would develop. Behring made antitoxin for tetanus. He received Noble Prize in 1901 for his work on serum therapy.

Pasteur isolated the bacterium responsible for chicken cholera and grew it in pure culture. He has taken two batches of chickens and he inoculated one batch with attenuated cultures (cultures of several weeks old) and the other batch with virulent (fresh culture) cultures. The batch that was inoculated with attenuated cultures developed resistance and the other batch died. He termed these attenuated cultures as Vaccines. Attenuated cultures stimulated hosts to develop antibodies. Pasteur later used this technique to prevent anthrax.

Pasteur's fame was well known throughout France and was asked to work on human disease 'Rabies' (Viral disease due to mad dog). He worked on it and prepared Vaccine to rabies.

Elie Metchnikoff described how certain leukocytes (white blood cells) could ingest (eat) disease producing bacteria. He called these special defenders against infection as Phagocytes (eating cells) and the process 'Phagocytosis', which is the first and most important defence against infection.

Paul Ehrlich first discovered the chemotherapeutic substance.

1880-1900 was the golden period for microbiology.

Another golden era of microbiology started about 1945 with foundation of modern biology i.e. Molecular Biology laid with the knowledge gained with different microorganisms.

EVOLUTION OF MICROBIOLOGY

Microbiology is a branch of science that deals with the study of diverse groups of microorganisms that include bacteria, fungi, algae and protozoa. It also includes viruses and acellular components that are sometimes considered non-living. The study of microbiology deals with the nutrition and functioning of microorganisms as well as their effect on plants and animals.

Microbiology has been derived from Greek words micros (small), bios (life) and logos (science). The name microbiology indicates that it includes microorganisms that cannot be seen with the naked eye. However there are some microbes that can be seen with naked eyes such as filamentous algae, bread moulds, mushrooms, and some bacteria.

Microbiology talks about how microbial life affects other microbes as well as non-microbial life. It encompasses the activities of microorganisms that are both beneficial and harmful to soil, water, plants, humans and animals and also the food we eat.

Microorganisms, also called microbes, are sometimes pathogenic in nature, i.e., they can cause diseases. However, very few microorganisms are pathogens. They are crucial life forms on earth that find applications in industrial, medicinal and agricultural fields.

It would not have been possible to study the vast field of microorganisms without the discovery of microscopes. Microscope is an optical instrument that magnifies small objects which could otherwise be not seen with naked eyes. It was discovered in the 17th century and is of two types: simple microscope and Compound microscope

Most of the microorganisms are unicellular, meaning they perform all their cellular functions in a single cell while some are multicellular. They can be prokaryotic in nature, meaning they do not have a membrane-bound nucleus or they can also be eukaryotic, having a membrane-bound nucleus.

The evolution of microorganisms has been in process since 2 billion years ago. The majority of life forms on the Earth are microbial. Studying the evolution of microbiology will help in understanding the origin of other life forms. The evolution of microbiology and microorganisms is an ongoing process because microbes are one life form that have a greater possibility of genetic variation, by methods such as genome reduction and horizontal gene transfer.

CLASSIFICATION

Three kingdom classification system

The configuration of organisms into taxonomic groups known as taxa based on similarities or relationships is known as classification. Closely related organisms (those with similar characteristics) are classified as belonging to the same taxon. The similarities and differences between organisms are used to classify them into larger groups. Because the categorization of living organisms is a complex and contentious subject, various taxonomic classification schemes have existed at various times. Linnaeus recognized only two kingdoms of living things in his classification scheme: Animalia and Plantae. Microscopic organisms had not been thoroughly studied at the time. They were either classified as a separate section titled Chaos or, in some cases, as plants or animals.

As understanding of the properties of various groups of microbial life grew, it became clear that a division of the living world into two kingdoms could not be justified on logical and consistent grounds at this level of biological knowledge. Then, in the 1860s, the German researcher Ernst Haeckel proposed a three-kingdom classification system.

The three-kingdom classification

Organisms lacking morphological complexities, a tissue system, division of labour, and a variety of feeding modes were separated and classified as Protista (algae, fungi, protozoa and bacteria).

Organisms with diverse tissue systems, a well-defined division of labour, and the greatest morphological complexities in their body were separated from protists and divided into two categories: those with an autotrophic mode of nutrition were considered plants and placed in the kingdom Plantae, while those with an entirely holophagic (phagotrophic) method of nutrition were considered animals and placed in the kingdom Animalia. According to this system, all known microorganisms were classified as protists; neither plants nor animals were classified as protists.

Kingdom Animalia

We are members of the Class Mammalia, which is part of the Phylum Chordata of the Kingdom Animalia. The Kingdom Animalia is a vast kingdom with numerous species. There were approximately 1.2 million animal species in the animal kingdom. Animals are classified based on their level of organisation, body symmetry, germ layers, coelom nature, segmentation, notochord, and so on.

Phylum Porifera, Hemichordata, Nematoda, Annelida, Arthropoda, Mollusca, Coelenterata (Cnidaria), Platyhelminthes, Echinodermata, and Chordata are the different phylum of the Kingdom Animalia. Animalia is distinguished by multicellular, eukaryotic animal forms. Metazoa is another

name for it. It is home to approximately 1.2 million animal species ranging from sponges to mammals.

Kingdom Plantae

Plants of various kinds surround us. The leaves, stems, flowers, and fruits of each plant help to identify it. They thrive in a variety of environments. Do you know why they don't resemble each other? There are many different kinds of plants on the planet, each with its own set of morphological and anatomical characteristics, as well as its own reproductive process.

All plants in the Kingdom Plantae are classified based on their characteristics. Kingdom Plantae is a large group of plants that are divided into subgroups. It is critical to understand plants, their functions, and characteristics. Continue reading to learn more about Kingdom Plantae.

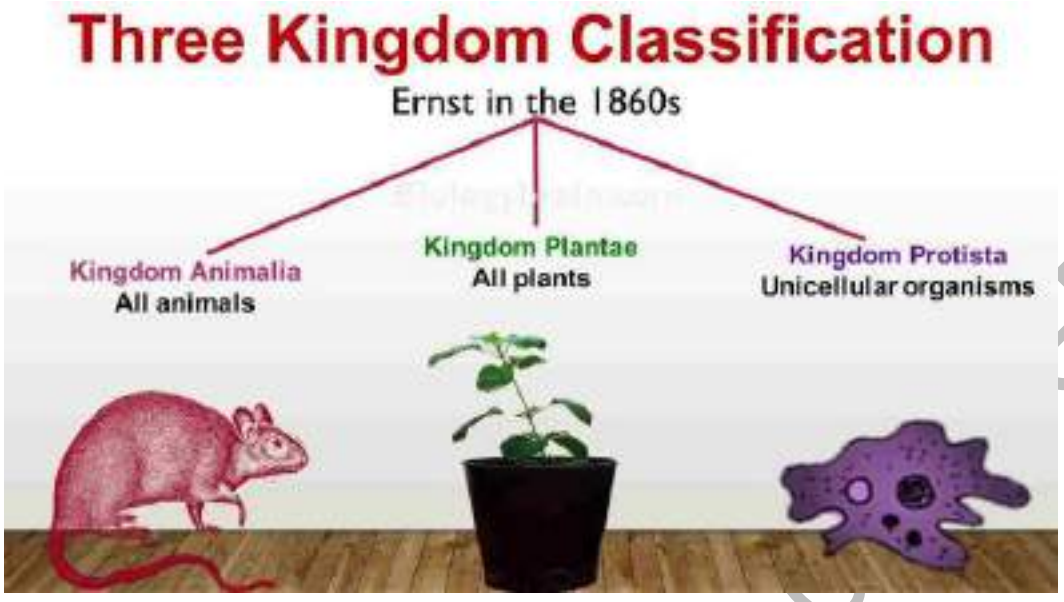
Kingdom Protista

Eukaryotic protists make up the Kingdom Protista. Members of this diverse kingdom are typically unicellular in structure and less complex than other eukaryotes. On the surface, these organisms are frequently described in terms of their similarities to the other groups of eukaryotes: animals, plants, and fungi.

Protists have few commonalities but are clustered together since they do not fit into any of the other kingdoms. Some protists can photosynthesise; some have mutualistic connections with other protists; a few are single celled; some are multicellular or form colonies; some of these are microscopic; some are enormous (giant kelp); some of these are bioluminescent; and some cause a variety of diseases in plants and animals.

Conclusion

Given the foregoing, efforts were made to find a solution, which was proposed in 1866 by E. Haeckel, a German zoologist and Darwin's disciple. Haeckel proposed that the inconsistencies of the two-kingdom system could be avoided by recognizing a third kingdom, and he proposed Protista as a new kingdom to accommodate organisms with characteristics that are either common to both plants and animals, or unique to them. As a result, the kingdoms Protista, Plantae, and Animalia evolved into a three-kingdom system. Haeckel made this arrangement based on morphological complexities and tissue systems, division of labour, and mode of nutrition.



The Five Kingdom Classification

Earlier, the organisms were classified into just two kingdoms- the classification of kingdom Plantae and animals. The classification of plants kingdom included every living organism that did not eat or move and grow continuously throughout their lives. The animal kingdom during this time included organisms that moved, ate, and stopped growing after a specific size.

R. H. Whittaker proposed the Five Kingdom Classification, and this classification made it easier to classify organisms into five different kingdoms-

1. Monera
2. Protista
3. Fungi
4. Plantae
5. Animalia

Classification basis for Five Kingdom Classification

The five kingdoms in this widely accepted classification consist of species of similar characteristics concerning their growth and functioning. The organisms are divided into the five kingdoms based on their general features like:

1. **Cell type:** Organisms can have either prokaryotic cells (cells lacking membrane) and be prokaryotes or have eukaryotic cells (a membrane covers genetic material) and be eukaryotes. The kingdom Monera is the only kingdom that consists of prokaryotes, as the other four kingdoms have eukaryotic organisms.

2. **Cell wall:** The kingdoms Monera, Fungi, and Plantae consist of organisms that have cell walls in their cells. Some of the Protists also have cell walls. But, the cells of the organisms of the Animalia kingdom do not have cell walls.
3. **Nuclear membrane**: The organisms under the Protists kingdom have cells without a nuclear membrane, while the others have a nuclear membrane.
4. **Cell organization:** This characteristic divides organisms into unicellular and multicellular.
5. **Nutrition mode:** The Plantae kingdom consists of autotrophs, i.e., they make their food. Fungi and Animalia kingdom consist of heterotrophs, i.e., organisms under this kingdom depend on others for food. Monera and Protista consist of both- autotrophs and heterotrophs.

Monera

The kingdom Monera of the five classification system categorizes bacteria underneath it has the following characteristics:

1. It contains all microscopic living organisms.
2. Kingdom Monera groups the prokaryotes together.
3. The monerans can be found in all habitats.
4. They are single-celled organisms with an absence of a well-defined nucleus.
5. The cells of monerans have cell walls formed from amino acids and polysaccharides.
6. The mode of nutrition of monerans can be either autotrophic or heterotrophic.

Based on their shape, bacteria are divided into four categories- cocci, bacilli, spirilla, and vibrio.

Protista

The general characteristics:

1. The cell type of the Protists is eukaryotic.
2. The organisms under Protista are unicellular.
3. These organisms usually use cilia, flagella, or amoeboid movement to move.
4. It has both autotrophs and heterotrophs.
5. The presence of a nuclear membrane marks the kingdom.
6. Some Protists have cell walls.
7. Cell fusion or zygote formation is used for sexual reproduction by Protists.

The kingdom Protista is further divided into Chrysophytes, Dinoflagellates, Euglenoids, Slime moulds, and Protozoans.

Fungi

General characteristics of Fungi are:

1. The kingdom contains eukaryotes.
2. A non-cellulosic cell wall made of chitin and polysaccharides is present in species of this kingdom.
3. A nuclear membrane is present in the organism's cells.
4. Fungi consist of heterotrophs, which can be parasitic or saprophytes.
5. Few Fungi are classified as symbionts. The symbionts living with algae are called lichens. At the same time, the ones living in association with higher plants are known as mycorrhiza.
6. Fungi species contain slender hyphae with long thread-like constructions. The mycelium is a web of hyphae.
7. Some hyphae are unbroken tubes with multinucleated cytoplasm called coenocytic hyphae.

Plantae

The features of the classification of plants kingdom are:

1. The classification of kingdom Plantae consists of eukaryotes that have chloroplasts.
2. The kingdom mostly has autotrophs, but there are certain exceptions.
3. Plants have a cellulosic cell wall with the presence of a nuclear membrane.
4. They are multicellular.
5. The diploid saprophytic and the haploid gametophytic phases have two different life phases.

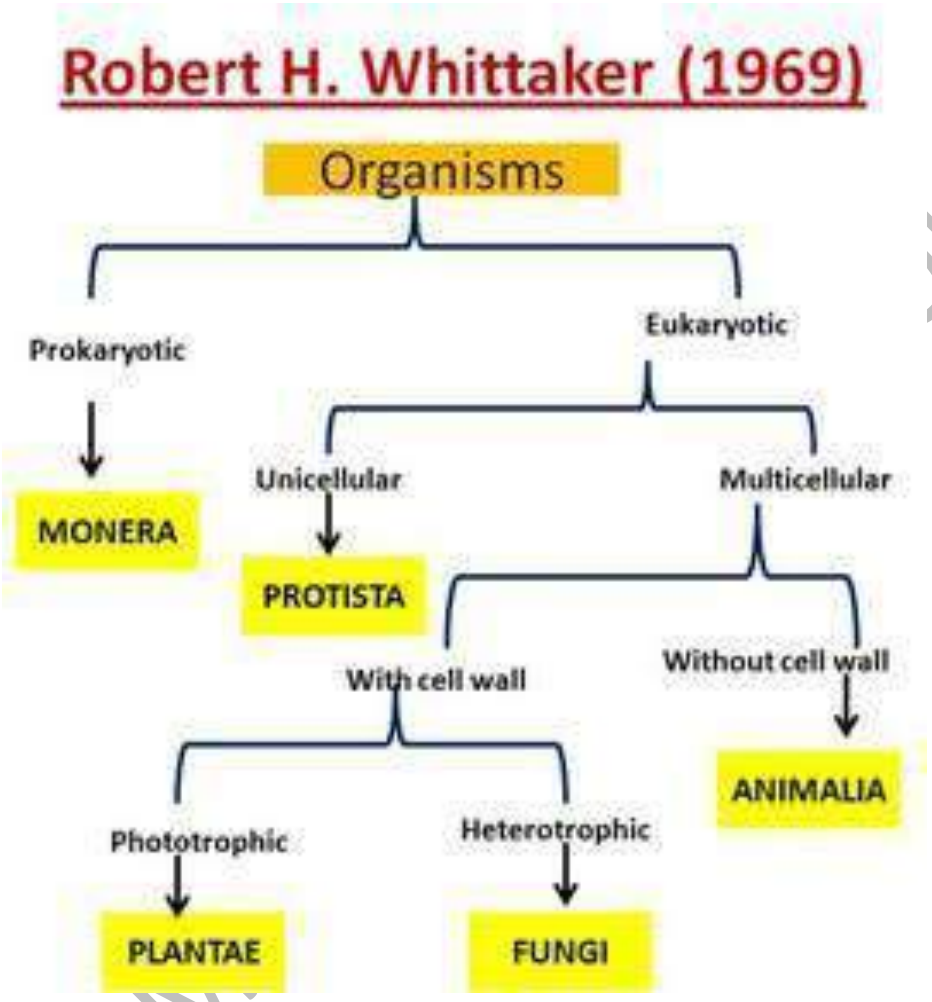
Animalia

This kingdom has the following features:

1. Animals are eukaryotes.
2. They do not have cell walls.
3. Unlike the classification of kingdom Plantae, there are heterotrophs underneath the Animalia kingdom.
4. Most species of this kingdom are adept at locomotion.
5. The reproduction mode for animals is sexual.

Conclusion

Before R, H, and Whittaker, numerous biologists proposed their classification systems. The Five Kingdom Classification system divides the organisms into five kingdoms- Monera, Protista, Fungi, Plantae, and Animalia.



The Six Kingdoms

When Linnaeus developed his system of classification, there were only two kingdoms, Plants and Animals. But the use of the microscope led to the discovery of new organisms and the identification of differences in cells. A two-kingdom system was no longer useful. Today the system of classification includes six kingdoms.

The Six Kingdoms:

Plants, Animals, Protists, Fungi, Archaebacteria, Eubacteria.

Plants

You are probably quite familiar with the members of this kingdom as it contains all the plants that you have come to know – flowering plants, mosses, and ferns. Plants are all multicellular and consist of complex cells.

In addition plants are autotrophs, organisms that make their own food. With over 250,000 species, the plant kingdom is the second largest kingdom. Plant species range from the tiny green mosses to giant trees.

Without plants, life on Earth would not exist! Plants feed almost all the heterotrophs (organisms that eat other organisms) on Earth.

Animals

The animal kingdom is the largest kingdom with over 1 million known species

All animals consist of many complex cells. They are also heterotrophs.

Members of the animal kingdom are found in the most diverse environments in the world.

Archaebacteria

In 1983, scientists tool samples from a spot deep in the Pacific Ocean where hot gases and molten rock boiled into the ocean form the Earth's interior. To their surprise they discovered unicellular (one cell) organisms in the samples. These organisms are today classified in the kingdom, Archaebacteria.

Archaebacteria are found in extreme environments such as hot boiling water and thermal vents under conditions with no oxygen or highly acid environments.

Finding Archaebacteria: The hot springs of Yellowstone National Park, USA, were among the first places Archaebacteria were discovered. The biologists pictured above are immersing microscope slides in the boiling pool onto which some archaebacteria might be captured for study.

Eubacteria

Like archaebacteria, eubacteria are complex and single celled. Most bacteria are in the Eubacteria kingdom. They are the kinds found everywhere and are the ones people are most familiar with.

Eubacteria are classified in their own kingdom because their chemical makeup is different.

Most eubacteria are helpful. Some produce vitamins and foods like yogurt. However, these eubacteria, Streptococci , can give you strep throat!

Fungi

Mushrooms, mould and mildew are all examples of organisms in the kingdom fungi. Most fungi are multicellular and consists of many complex cells. Some fungi taste great and others can kill you!

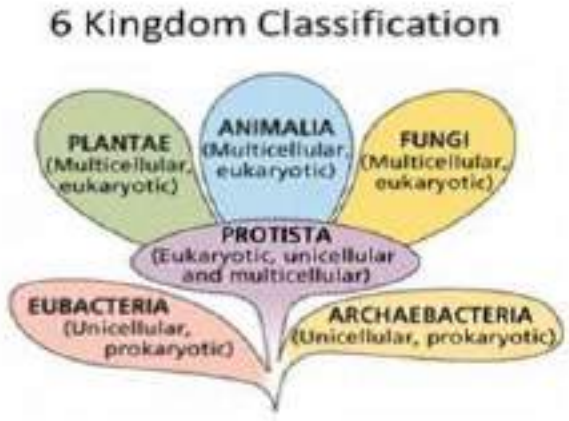
Fungi are organisms that biologists once confused with plants, however, unlike plants, fungi cannot make their own food. Most obtain their food from parts of plants that are decaying in the soil.

Protists

Slime moulds and algae are protists.

Sometimes they are called the odds and ends kingdom because its members are so different from one another. Protists include all microscopic organisms that are not bacteria, not animals, not plants and not fungi.

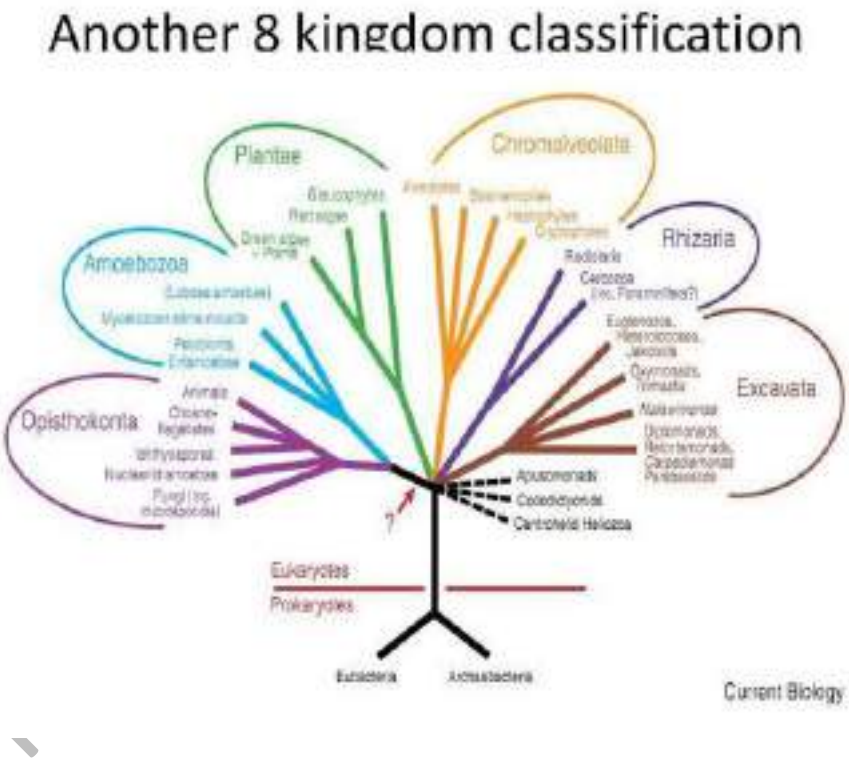
Most protists are unicellular. You may be wondering why those protists are not classified in the Archaebacteria or Eubacteria kingdoms. It Is because, unlike bacteria, protists are complex cells. These delicate looking diatoms are classified in the protist kingdom.



EIGHT KINGDOM CLASSIFICATION

The eight Kingdom system is propose by Cavalier Smith in 1987. This concept is based on cellular structure and genetic organisation. He used ultrastructural characteristics as well as RNA sequences. He divides all organisms into two empires and eight kingdoms. Two empires in this concept are bacteria and eukaryota. The empire Bacteria contains two kingdoms they are Eubacteria and the Archaeobacteria. The second empire, the Eucaryota, contains six kingdoms they are Archezoa, Protozoa , Plantae , Chromista, Fungi , Animalia. The Archezoa are primitive eukaryotic unicellular organisms such as Giardia that have 70S ribosomes and lack Golgi apparatus, mitochondria, chloroplasts and peroxisomes.

The kingdom Chromista contains mainly photosynthetic organisms that have their chloroplasts within the lumen of the rough endoplasmic reticulum. Eg. Diatoms, brown algae, cryptomonads and oomycetes.



INTRODUCTION TO BIODIVERSITY

Microbial diversity' considers the vast array of microorganisms—the smallest forms of life—which exist everywhere. The three primary groups of microorganisms are bacteria, archaea, and eukaryotes. Bacteria and archaea are prokaryotes with their genetic material held in a single chromosome. In eukaryotes, most of the genome is held in multiple chromosomes. Over 11,000 species of bacteria have been identified using microscopic identification of cell shape and metabolic activity, Gram-staining techniques, and genetic identification of RNA and DNA sequences. There are 500 named species of archaea, divided into two phyla: the euryarchaeota and the crenarchaeota. There are eight super groupings of eukaryotes, all of them include single-celled organisms, and five are entirely microbial.

ECOLOGICAL NICHE

Microbial life is amazingly diverse and microorganisms quite literally cover the planet. In fact, it has been estimated that there are 100,000,000 times more microbial cells on the planet than there are stars in the observable universe! Microbes live in all parts of the biosphere where there is liquid water, including soil, hot springs, the ocean floor, acid lakes, deserts, geysers, rocks, and even the mammalian gut.

By virtue of their omnipresence, microbes impact the entire biosphere; indeed, microbial metabolic processes (including nitrogen fixation, methane metabolism, and sulfur metabolism) collectively control global biogeochemical cycling. The ability of microbes to contribute substantially to the function of every ecosystem is a reflection their tremendous biological diversity.

Microbes are vital to every ecosystem on Earth and are particularly important in zones where light cannot approach (that is, where photosynthesis cannot be the basic means to collect energy). Microorganisms participate in a host of fundamental ecological processes including production, decomposition, and fixation. They can also have additional indirect effects on the ecosystem through symbiotic relationships with other organisms. In addition, microbial processes can be co-opted for biodegradation or bioremediation of domestic, agricultural, and industrial wastes, making the study of microbial ecology particularly important for biotechnological and environmental applications.

Each species in an ecosystem is thought to occupy a separate, unique niche. The ecological niche of a microorganism describes how it responds to the distribution of resources and competing species, as well as the ways in which it alters those same factors in turn. In essence, the niche is a complex description of the ways in which a microbial species uses its environment.

The precise ecological niche of a microbe is primarily determined by the specific metabolic properties of that organism. For example, microbial organisms that can obtain energy from the oxidation of inorganic compounds (such as iron-reducing bacteria) will likely occupy a different niche from those that obtain energy from light (such as cyanobacteria). Even among photosynthetic bacteria, there are various species that contain different photosynthetic pigments (such as chlorophylls and carotenoids) that allow them to take advantage of different portions of the electromagnetic spectrum; therefore, even microbes with similar metabolic properties may inhabit unique niches.

BASIC CONCEPTS OF EUBAPCTERIA

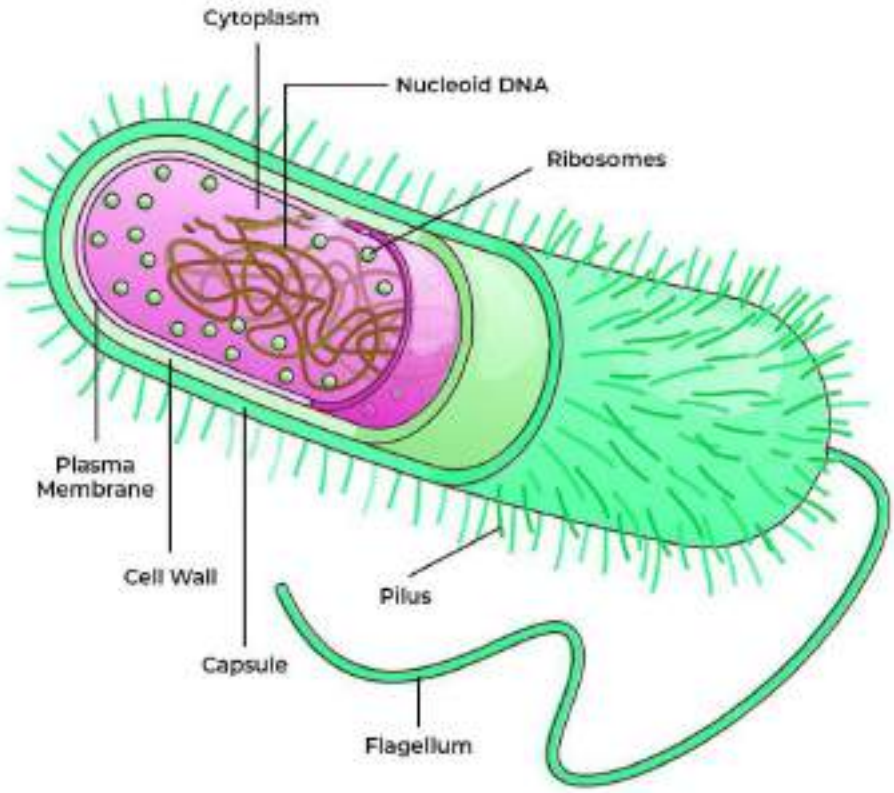
They are aka 'True bacteria'. Eubacteria are prokaryotic organisms (i.e. lacking a membrane-bound nucleus), predominantly unicellular and single cellular DNA chromosomes. They have peptidoglycan in their cell wall and usually have flagella if they are motile. It is one of the three domains in the three-domain system of classification proposed by Woese. They can be found in a variety of environments around the world. Almost all kinds of bacteria fall under this, except archaebacteria.

Characteristics

- True bacteria is also the name given to eubacteria. These are prokaryotic organisms that are single-celled.
- Eubacteria have stiff cell walls due to peptidoglycans.
- For locomotion they have flagella.
- The majority of Eubacteria are heterotrophic. However, a few are photosynthetic or chemosynthetic.
- Some bacteria have pili, which are small appendages present on the surface of the cell which assists in sexual reproduction. Pili also helps in the attachment of pathogens to their hosts.
- The size of these bacteria ranges from 0.2 to 50 micrometres.
- Depending on the type of cell wall and the gram stain they take, they are classified as gram-positive or gram-negative. The gram-negative bacteria do not take the gram stain and are harmful to humans. Whereas the gram-positive bacteria take up the gram stain and are beneficial to human health.

Structure of Eubacteria

The structure of the eubacterial cell can be described as follows:



The outermost layer of the cell is the cell wall which is made up of peptidoglycan. In some bacteria, additionally, the capsule is present which is made up of sugars and proteins. Many eubacteria have a cellular appendage called flagella that allows them to move through rotational motion. These flagella are made up of flagellin proteins and extend out from the cell wall. The base of the flagella is fixed within the cell membrane.

The cell wall is followed by the cell membrane and it is made up of a bilayer of phospholipids, proteins, and sugars. Plasma membrane is a selectively permeable i.e not all particles cross the membrane. Enclosed within the cell membrane is cytoplasm in which cellular contents are present. Eubacteria lack a well-developed nucleus and other membrane-bound organelles. DNA is present as the naked and coiled structure in the cytoplasm, such a structure is called the nucleoid. Eubacteria also have extrachromosomal circular DNA called plasmids which give them resistance to antibiotics and also provide pathogenicity. In the cytoplasm, enzymes and proteins are present to carry out metabolic activities. Sometimes, invaginations of the cell membrane are seen in the cytoplasm called mesosomes. These contain photosynthetic and respiratory enzymes.

Types of Eubacteria

On the basis of shape they are of four types:

Coccus: These bacteria have a spherical shape. Eg: Staphylococcus, Pneumococcus.

Bacillus: These are rod-shaped bacteria. Eg: Anthracis, B. megaterium and B. thuringiensis.

Vibrio: These are comma-shaped bacteria. Eg: V. cholera, V. parahaemolyticus, and V. vulnificus.

Spirillum: These are spiral-shaped bacteria. Eg: Spirochaete, Treponema, Borrelia, and Leptospira.

On the basis of the stain they take during gram staining developed by Christian Gram, they are classified into two types:

Gram-positive bacteria: These bacteria take up the gram stain and are not pathogenic.
Eg: Staphylococcus, Streptococcus, etc.
Gram-negative bacteria: These bacteria do not take up the gram stain and are pathogenic.
Eg: E. coli, Salmonella

Classification of Eubacteria

There are basically two main types of Eubacteria which are as follows:

Cyanobacteria

They are aka BGA (Blue Green Algae). They are gram-negative bacteria. Cyanobacteria are the most primitive organisms to exhibit oxygenic photosynthesis. They possess chlorophyll similar to higher plants. They are mostly found in freshwater though a few are found in marine environments. They show symbiosis with almost all eukaryotic groups. Eg: Anabaena is found associated with the coralloid roots of Cycas. Their body structure is varied. They can be either unicellular, filamentous, or colonial. They may possess trichomes. However, flagella are strictly absent in them. Cyanobacteria help in nitrogen fixation as they contain nitrogenase-containing heterocyst Eg Nostoc. They reproduce asexually. True sexual reproduction is absent in them.

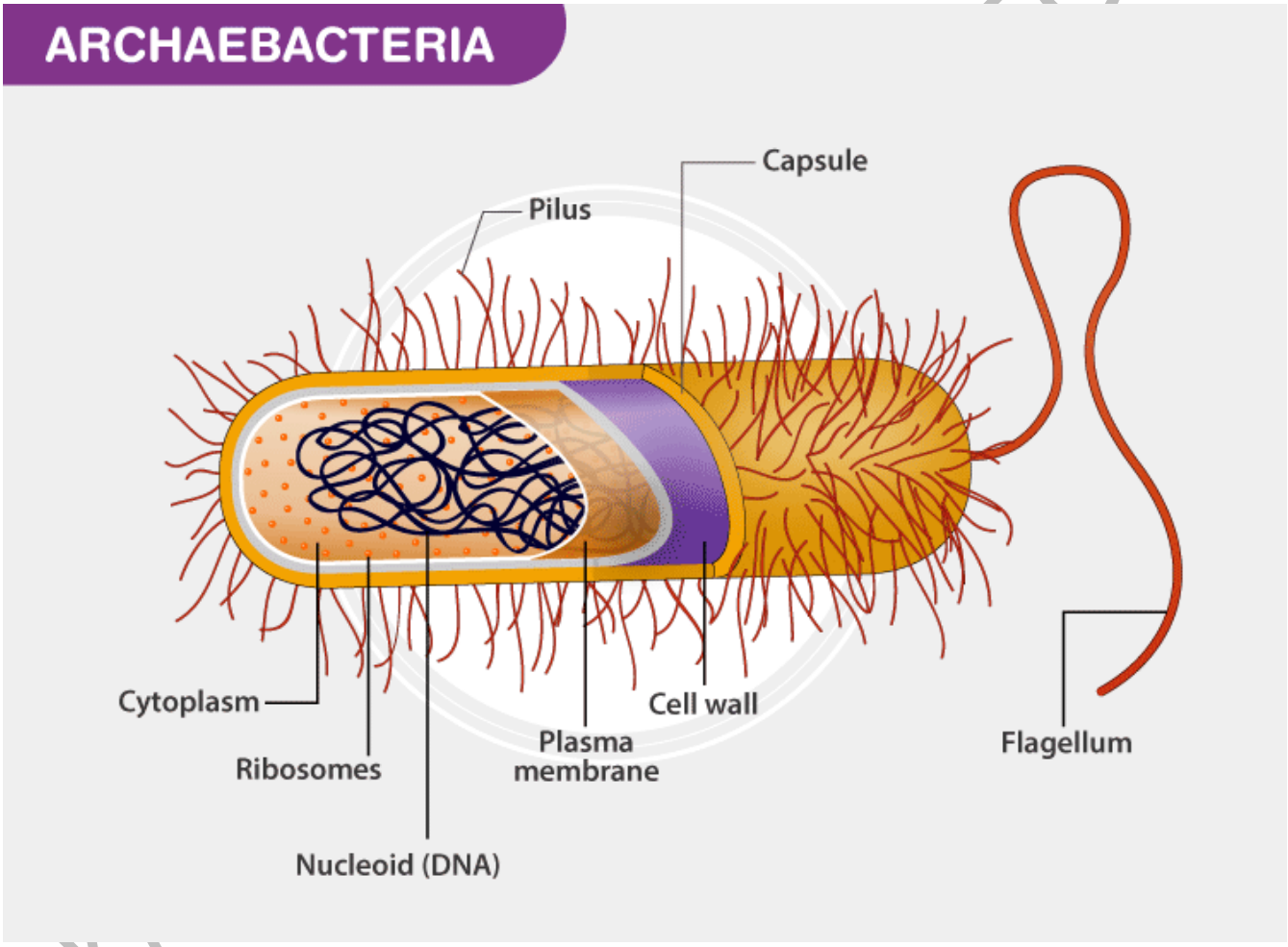
Mycoplasma

They exist in various shapes and hence are called PPLO (Pleuropneumonia Like Organisms). They lack true cell walls and hence are called ‘bacteria with their coats off and ‘jokers of the plant kingdom’. PPLO are the smallest living organisms with a size of about 0.02-0.2 microns. They are facultative anaerobes. They exhibit a heterotrophic mode of nutrition and are generally parasitic. Some are saprotrophs. They are pathogenic to both plants and animals. Mycoplasma possesses resistance to antibiotics like Penicillin and Bacitracin. However, they lack resistance to antibiotics such as Streptomycin, Chloramphenicol, and Tetracycline. Mycoplasma can pass through bacteriological filters due to its extremely small size.

BASIC CONCEPTS OF ARCHAEBACTERIA

Archaebacteria are known to be the oldest living organisms on earth. They belong to the kingdom Monera and are classified as bacteria because they resemble bacteria when observed under a microscope. Apart from this, they are completely distinct from prokaryotes. However, they share slightly common characteristics with the eukaryotes.

These can easily survive under very harsh conditions such as the bottom of the sea and the volcanic vents and are thus known as extremophiles.



Archaebacteria

Characteristics of Archaebacteria

Following are the important characteristics of archaebacteria:

- Archaebacteria are obligate or facultative anaerobes, i.e., they flourish in the absence of oxygen and that is why only they can undergo methanogenesis.
- The cell membranes of the Archaebacteria are composed of lipids.
- The rigid cell wall provides shape and support to the Archaebacteria. It also protects the cell from bursting under hypotonic conditions.
- The cell wall is composed of Pseudomurein, which prevents archaebacteria from the effects of Lysozyme. Lysozyme is an enzyme released by the immune system of the host, which dissolves the cell wall of pathogenic bacteria.
- These do not possess membrane-bound organelles such as nuclei, endoplasmic reticulum, mitochondria, lysosomes or chloroplast. Its thick cytoplasm contains all the compounds required for nutrition and metabolism.
- They can live in a variety of environments and are hence called extremophiles. They can survive in acidic and alkaline aquatic regions, and also in temperature above boiling point.
- They can withstand a very high pressure of more than 200 atmospheres.
- Archaebacteria are indifferent towards major antibiotics because they contain plasmids which have antibiotic resistance enzymes.
- The mode of reproduction is asexual, known as binary fission.
- They perform unique gene transcription.
- The differences in their ribosomal RNA suggest that they diverged from both prokaryotes and eukaryotes.

Types of Archaebacteria

Archaebacteria are classified on the basis of their phylogenetic relationship. The major types of Archaebacteria are discussed below:

Crenarchaeota

The Crenarchaeota are Archaea, which exist in a broad range of habitats. They are tolerant to extreme heat or high temperatures. They have special proteins that help them to function at temperatures as high as 230 degrees Celsius. They can be found in deep-sea vents and hot springs, regions with superheated water. These include thermophiles, hyperthermophiles, and thermoacidophiles.

Euryarchaeota

These can survive under extremely alkaline conditions and have the ability to produce methane, unlike any other living being on earth. These include methanogens and halophiles.

Korarchaeota

They possess the genes common with Crenarchaeota and Euryarchaeota. All three are believed to have descended from a common ancestor. These are supposed to be the oldest surviving organism on earth. These include hyperthermophiles.

Thaumarchaeota

These include archaea that oxidize ammonia.

Nanoarchaeota

This is an obligate symbiont of archaea belonging to the genus Ignicoccus.

The importance of archaebacteria can be understood from the following points:

- Archaebacteria have compelled the scientists to reconsider the common definition of species. Species are a group with gene flow within its members. The archaebacteria exhibit gene flow across its species.
- The Archaebacteria are methanogens, i.e., they are capable of producing methane. They act on the organic matter and decompose it to release methane which is then used for cooking and lighting.

Examples of Archaebacteria

Following are the important examples of archaebacteria:

Lokiarcheota

It is a thermophilic archaebacterium found in deep-sea vents known as the Loki's castle. It has a unique genome. Some of the genes of the genome are involved in phagocytosis. They also possess the eukaryotic genes that are used by the eukaryotes to control their shapes. It is believed that Lokiarcheota and eukaryotes shared a common ancestor several billion years ago.

Methanobrevibacter smithii

It is a methane-producing bacteria found in the human gut. It helps in the breakdown of complex plant sugars and extracts energy from the food consumed by us. Some help to protect against colon cancer. People suffering from colon cancer and obesity have very high levels of Euryarchaeota bacteria in their gut.

The Archaebacteria cannot perform photosynthesis and show high levels of gene transfer between lineages. The discovery of Archaebacteria has made scientists believe that life can exist even in extreme environmental conditions.

BASIC CONCEPTS OF EUKARYA

Eukarya are essentially all cell-based organisms containing nuclei or membrane-bound organelles, divided into kingdoms of Plants, Animalia, Fungi, and a handful of Protista. Eukarya includes eukaryotic organisms. These are organisms with cells that contain a nucleus as well as membrane-bound organelles. The kingdoms most associated with Eukarya are the Plantae, Animalia, and Fungi kingdoms. Additionally, Kingdom Protista has had some of its organisms, such as amoebas and some seaweeds, classified as Eukarya.

We, as humans, are classified as Eukarya. All of our own cells have a nucleus and membrane-bound organelles. In fact, all complex organisms are eukaryotic. There is just no real way for prokaryotic organisms to grow into the complexity that eukaryotes have.

Eukarya represent only a small amount of living organisms, with prokaryotes outnumbering them by a large margin. Organisms in the Eukarya domain split through mitosis (cell division) and reproduce through meiosis (sexual reproduction where male and female gametes combine). Eukarya is a rather 'young' domain because eukaryotes only came about around 1.7 billion years ago. If we recall that the earth is roughly 4.6 billion years old, 1.7 seems rather young. The first prokaryotes, for example, showed up around 3.8 billion years ago.

CONSERVATION OF BIODIVERSITY

Strategies for Biodiversity Conservation

Following are the important strategies for biodiversity conservation:

1. All the varieties of food, timber plants, livestock, microbes and agricultural animals should be conserved.
2. All the economically important organisms should be identified and conserved.
3. Unique ecosystems should be preserved first.
4. The resources should be utilized efficiently.
5. Poaching and hunting of wild animals should be prevented.
6. The reserves and protected areas should be developed carefully.
7. The levels of pollutants should be reduced in the environment.
8. Deforestation should be strictly prohibited.
9. Environmental laws should be followed strictly.
10. The useful and endangered species of plants and animals should be conserved in their nature as well as artificial habitats.

11. Public awareness should be created regarding biodiversity conservation and its importance.

It is believed that an area with higher species abundance has a more stable environment compared to an area with lower species abundance. We can further claim the necessity of biodiversity by considering our degree of dependency on the environment. We depend directly on various species of plants for our various needs. Similarly, we depend on various species of animals and microbes for different reasons.

Biodiversity is being lost due to the loss of habitat, over-exploitation of resources, climatic changes, pollution, invasive exotic species, diseases, hunting, etc. Since it provides us with several economic and ethical benefits and adds aesthetic value, it is very important to conserve biodiversity.

UNIT – II

BACTERIA

Until the eighteenth century all the living organisms were grouped into two kingdoms, plant and animal. After the discovery of the microbial world, it is evident that some organisms are predominantly plant like, some are animal like and some others share the characteristics common to both plants and animals. Since there are organisms that do not fall into either plant or animal kingdom, it was proposed that new kingdom be established to include those organisms, which typically are neither plants nor animals. E.H. Haeckel in 1866 proposed a third kingdom '**Protista'** to include the microorganisms that are typically neither plants nor animals. Bacteria, algae, fungi and protozoa are included in **Protists** (Viruses are not cellular organisms and hence not classified as protists). Bacteria are referred to as **lower protists**, whereas the fungi, algae and protozoa are called **higher protists**.

The major characteristics of Bacteria are based on their size, shape and arrangements.

SIZE

The unit of measurement used in bacteriology is the micron (micrometre)

1 micron (μ) or micrometre (μm) – one thousandth of a millimetre

1 millimicron (mμ) or nanometre (nm) – one thousandth of a micron or one millionth of a millimetre

1 Angstrom unit (Å) – one tenth of a nanometre

The limit of resolution with the unaided eye is about 200 microns. Bacteria are smaller which can be visualized only under magnification. Bacteria of medical importance generally measure 0.2 – 1.5 μm in diameter and about 3-5 μm in length.

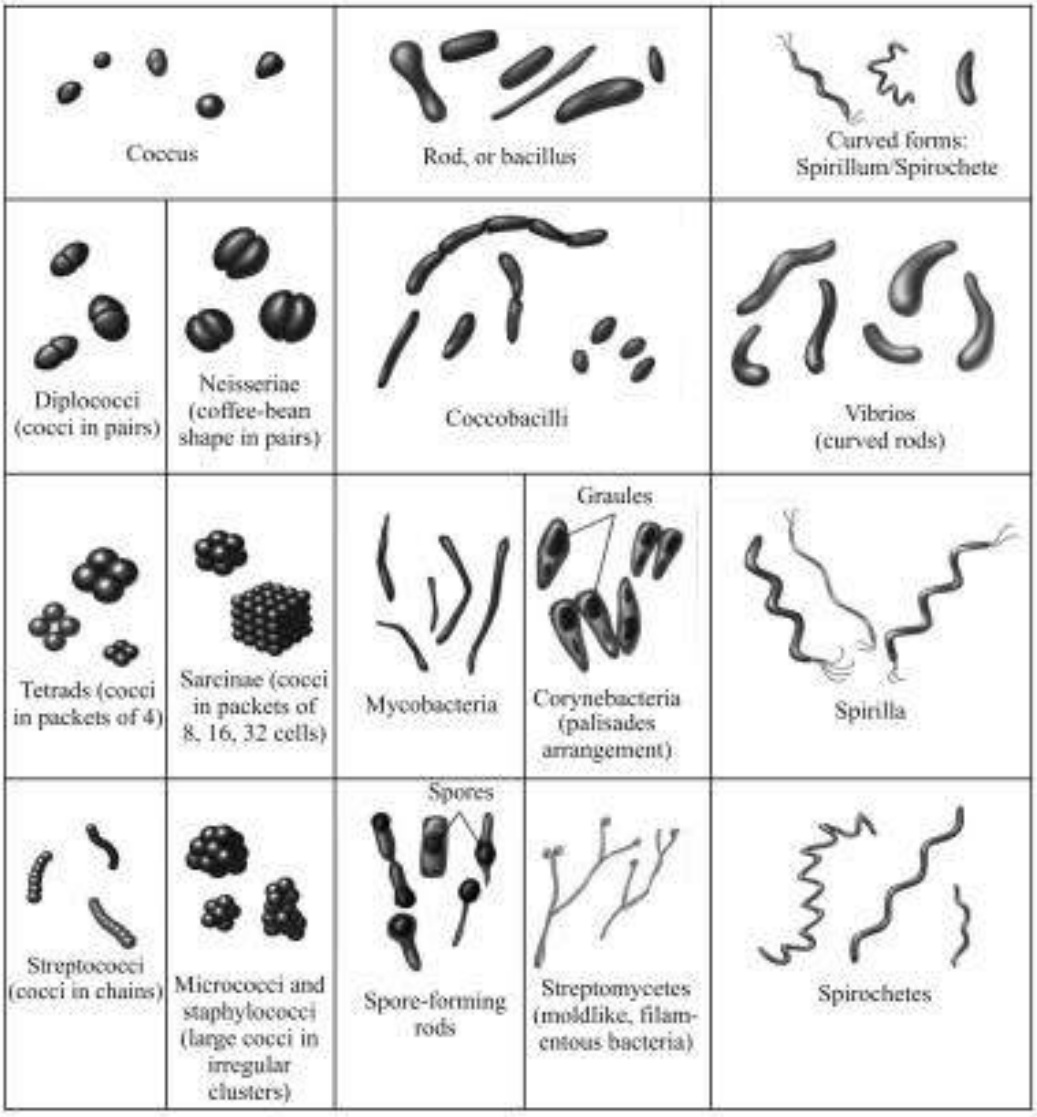
SHAPE OF THE BACTERIA

Depending on their shape, bacteria are classified into several varieties

- Cocci (from kokkos meaning berry) are spherical or oval cells
- Bacilli (from baculus meaning rod) are rod shaped cells
- Vibrio's are comma shaped curved rods and derive their name from their characteristics vibratory motility.
- Spirilla are rigid spiral forms.
- Spirochetes (from speira meaning coil and chaite meaning hair) are flexuous spiral forms

- Actinomycetes are branching filamentous bacteria, so called because of a fancied resemblance to the radiating rays of the sun when seen in tissue lesions (from actis meaning ray and mykes meaning fungus)
- Mycoplasmas are bacteria that are cell wall deficient and hence do not possess a stable morphology. They occur as round or oval bodies and as interlacing filaments.

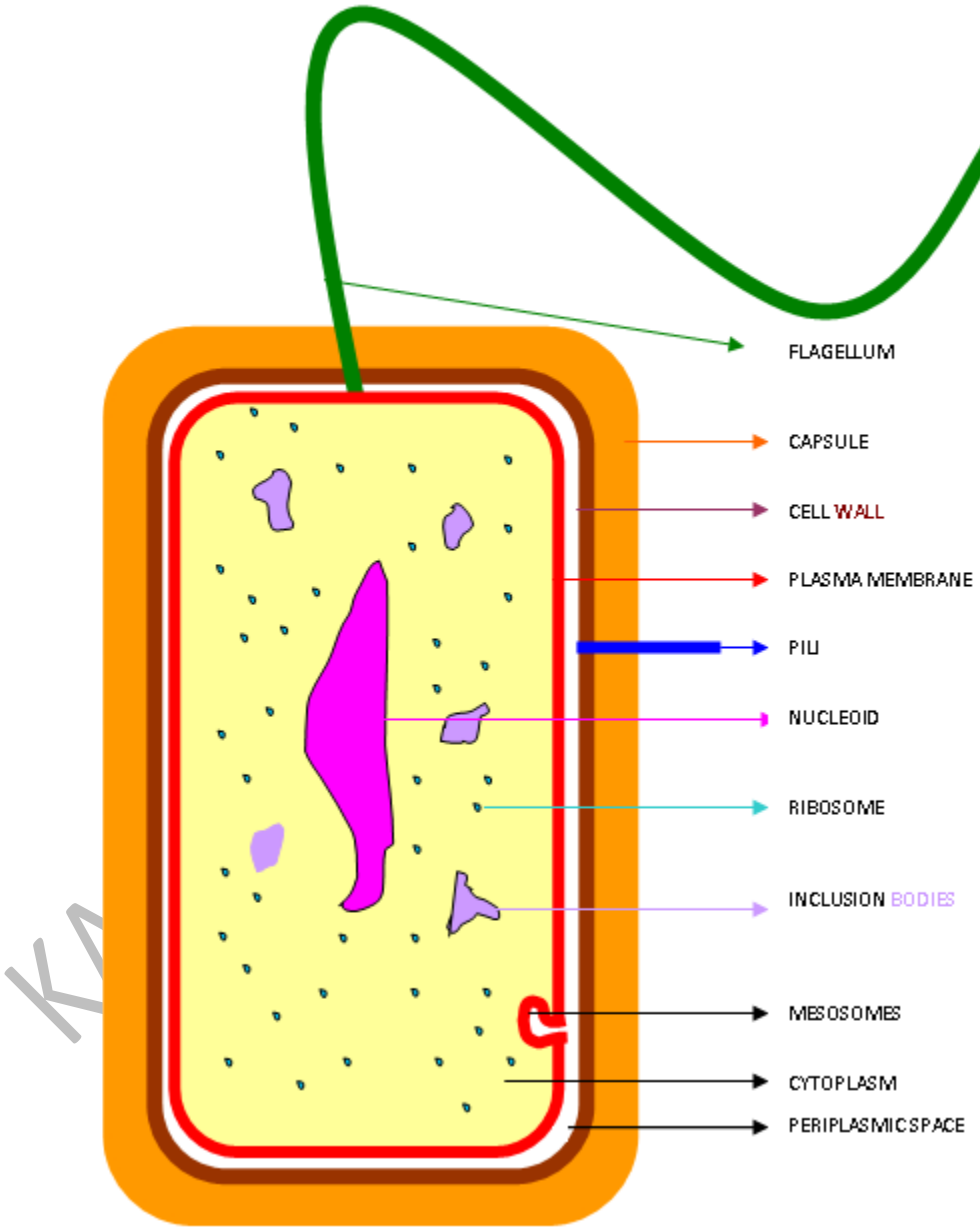
Bacteria sometime show characteristic cellular arrangement or grouping. According to the plane of cellular division, cocci may be arranged in pairs (diplococci), chains (streptococci), groups of four (tetrads) or eight (sarcina), or grape like clusters (staphylococci).



BACTERIAL STRUCTURE

The outer layer or cell envelope consists of two components, a rigid cell wall and beneath it a cytoplasmic or plasma membrane. The cell envelope encloses the protoplasm, comprising the cytoplasm, cytoplasmic inclusions such as ribosomes and mesosomes, granules, vacuoles and the nuclear body.

TYPICAL BACTERIAL CELL STRUCTURE AND FUNCTIONS OF DIFFERENT PARTS OF BACTERIAL CELLS



Cell wall

Beneath the external structures is the cell wall. It is very rigid & gives shape to the cell. Its main function is to prevent the cell from expanding & eventually bursting due to water uptake. Cell Wall constitutes a significant portion of the dry weight of the cell and it is essential for bacterial growth & division. The cell wall cannot be seen by direct light microscopy and does not stain with simple stains. It may be demonstrated by microdissection, reaction with specific antibodies, mechanical rupture of the cell, differential staining procedures or by electron microscopy.

Chemically the cell wall is composed of peptidoglycan. Mucopeptide (peptidoglycan or murien) formed by N acetyl glucosamine & N acetyl muramic acid alternating in chains, cross linked by peptide chains. Embedded in it are polyalcohol called Teichoic acids. Some are linked to Lipids & called Lipoteichoic acid. Lipotechoic acid link peptidoglycan to cytoplasmic membrane and the peptidoglycan gives rigidity.

The functions of Teichoic acid are

- gives negative charge
- major antigenic determinant
- transport ions
- anchoring
- external permeability barrier

Characteristics Gram Positive Gram Negative Thickness Thicker Thinner Variety of amino acids Few Several Lipids Absent Present Teichoic acid Present absent

Outer Membrane

Outer membrane is found only in Gram-negative bacteria, it functions as an initial barrier to the environment and is composed of lipopolysaccharide (LPS) and phospholipids

Lipopolysaccharide (LPS)

The LPS present on the cell walls of Gram-negative bacteria account for their endotoxic activity and antigen specificity.

A bacterium is referred as a protoplast when it is without cell wall. Cell wall may be lost due to the action of lysozyme enzyme, which destroys peptidoglycan. This cell is easily lysed and it is metabolically active but unable to reproduce.

A bacterium with a damaged cell wall is referred as spheroplasts. It is caused by the action of toxic chemical or an antibiotic, they show a variety of forms and they are able to change into their normal form when the toxic agent is removed, i.e. when grown on a culture media

Cytoplasmic membrane

Cytoplasmic membrane is present immediately beneath the cell wall, found in both Gram positive & negative bacteria and it is a thin layer lining the inner surface of cell wall and separating it from cytoplasm. It acts as a semipermeable membrane controlling the flow of metabolites to and from the protoplasm.

Cytoplasm

The cytoplasm is a Colloidal system containing a variety of organic and inorganic solutes containing 80% Water and 20% Salts, Proteins. They are rich in ribosomes, DNA & fluid. DNA is circular and haploid. They are highly coiled with intermixed polyamines & support proteins. Plasmids are extra circular DNA.

Ribosomes

They are the centers of protein synthesis. They are slightly smaller than the ribosomes of eukaryotic cells

Mesosomes

They are vesicular, convoluted tubules formed by invagination of plasma membrane into the cytoplasm. They are principal sites of respiratory enzymes and help with cell reproduction

Cytoplasmic Inclusions

The Inclusion bodies are aggregates of polymers produced when there is excess of nutrients in the environment and they are the storage reserve for granules, phosphates and other substances. Volutin granules are polymetaphosphates which are reserves of energy and phosphate for cell metabolism and they are also known as metachromatic granules.

Nucleus

The Nucleus is not distinct and has no nuclear membrane or nucleolus and the genetic material consist of DNA. The cytoplasmic carriers of genetic information are termed plasmids or episomes.

Capsule

Capsule is the outer most layer of the bacteria (extra cellular). It is a condensed well defined layer closely surrounding the cell. They are usually polysaccharide and if polysaccharide envelops the whole bacterium it is capsule and their production depends on growth conditions. They are secreted by the cell into the external environment and are highly impermeable. When it forms a loose mesh work of fibrils extending outward from the cell they are described as glycocalyx and

when masses of polymer that formed appear to be totally detached from the cell and if the cells are seen entrapped in it are described as slime layer.

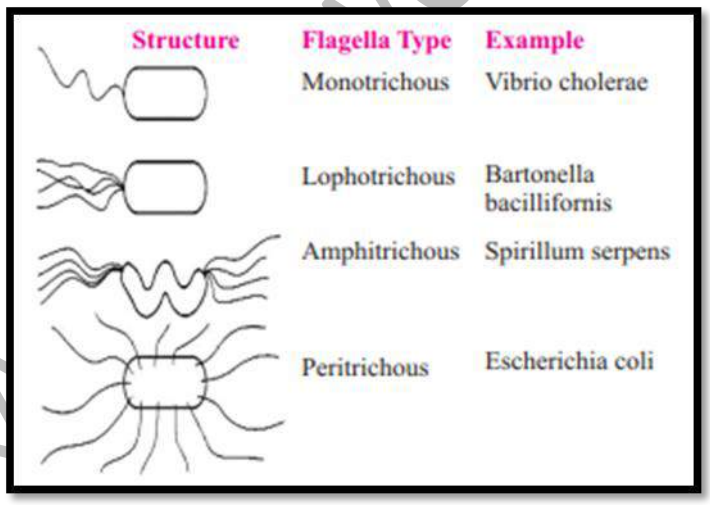
The Capsule protects against complement and is antiphagocytic. The Slime layer & glycocalyx helps in adherence of bacteria either to themselves forming colonial masses or to surfaces in their environment and they resists phagocytosis and desiccation of bacteria.

Flagella

Flagella are long hair like helical filaments extending from cytoplasmic membrane to exterior of the cell. Flagellin is highly antigenic and functions in cell motility. The location of the flagella depends on bacterial species as polar situated at one or both ends which swims in back and forth fashion and lateral at along the sides.

The parts of flagella are the filament, hook and the basal body. Filament is external to cell wall and is connected to the hook at cell surface, the hook & basal body are embedded in the cell envelope. Hook & filament is composed of protein subunits called as flagellin. Flagellin is synthesized within the cell and passes through the hollow centre of flagella. The arrangement of flagella may be described as

(i) Monotrichous – single flagella on one side
(ii) Lophotrichous – tuft of flagella on one side
(iii) Amphitrichous – single or tuft on both sides
(iv) Peritrichous – surrounded by lateral flagella



Various types of mobility is observed because of the presence of the flagella as Serpentine motility is seen with Salmonella, Darting motility with Vibrio and Tumbling motility with Listeria monocytogenes.

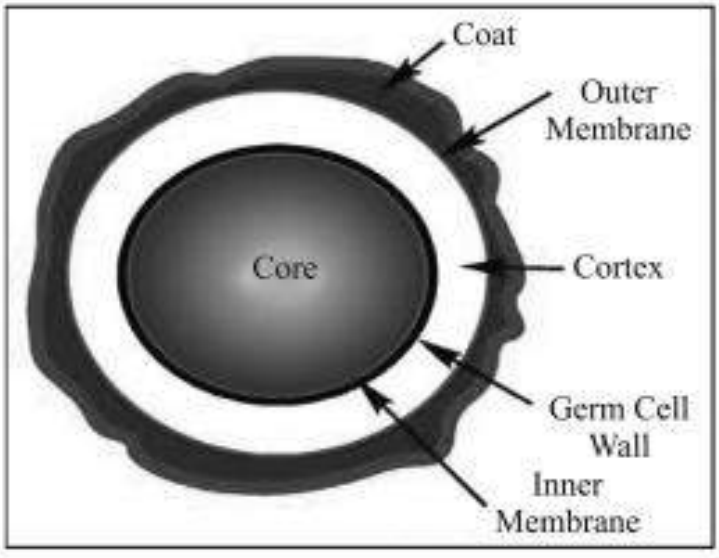
Pili / Fimbriae

Hair-like proteinaceous structures that extend from the cell membrane to external environment are pili which are otherwise known as fimbriae. They are thinner, shorter and more numerous than flagella and they do not function in motility. The fimbriae is composed of a subunit called pilin.

There are two types pili namely Non-sex pili (Common pili) eg. fimbriae or type IV and the sex pili. The fimbriae are antigenic and mediate their adhesion which inhibits phagocytosis. The sex pili help in conjugation.

Spore

Some bacteria have the ability to form highly resistant resting stage called spores, which helps them to overcome adverse environmental conditions that are unfavourable for vegetative growth of cell. They are not a reproductive form and not a storage granule. These spores are resistant to bactericidal agents and adverse physical conditions. Each spore can give rise to only one endospore which play a role in heat resistance. Spores consists of three layers namely core, cortex and spore coat.



Characteristics of Bacteria Cell Structures

Structure	Functions(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; protection against desiccation	Usually polysaccharide; occasionally polypeptide
Cell wall		
Gram-positive bacteria	confers rigidity and shape on cells	Peptidoglycan (murein) complexed with teichoic acids
Gram-negative bacteria	confers rigidity and shape; outer membrane is permeability barrier; associated LPS and proteins have various functions	Peptidoglycan (murein) surrounded by phospholipid protein-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

GRAM -vs BACTERIA

There are a number of important gram-negative bacteria that are not closely related to the gram-negative proteobacteria. They include several physiologically and morphologically distinctive photosynthesizing bacteria, such as those included in the phyla cyanobacteria, chlorobi (green sulphur bacteria) and chloroflexi (green non sulphur bacteria). The cyanobacteria produce oxygen during photosynthesis, and the green sulphur and green non-sulphur bacteria do not produce oxygen.

The gram-negative eubacteria include a number of species that are obligately anaerobic. The gram-negative fermentative bacteria are capable of fermenting a wide range of sugars, amino acids and other organic acids, some are capable of fumarate or nitrate linked respiration. They are characteristic symbionts within the alimentary tract of homeothermic animals.

1. Spirochete
2. Rickettsia
3. Chlamydia
4. Gliding Bacteria
5. Sheathed Bacteria
6. Chemolithotrophs
7. Anoxygenic and Oxygenic Phototrophic Bacteria
8. Cyanobacteria
9. Purple Bacteria
10. Green Bacteria
11. Budding Bacteria

GRAM +ve BACTERIA

The Gram-positive bacteria can be divided into two groups: those that have a high G + C ratio, and those that have a low G + C ratio. To illustrate the variations in G +C ratio, the genus Streptococcus has a low G + C content of 33-34% and the genus Clostridium has a low content of 21-54%.

By contrast, filamentous actinomycetes of the genus Streptomyces have a high G + C content of 69-73%. Gram-positive bacteria of a more conventional morphology, such as the genera Conjllebacterillm and Mycobacterium, have, respectively, a G + C content of 51-63% and 62-70%.

The Gram-positive bacteria contain a number of anaerobic or facultatively anaerobic organisms, some of these are members of the actinomycete group, and many of them are unicellular bacteria unrelated to the mycelial prokaryotes.

Many Gram positive bacteria share the ability to form a distinctive type of dormant cell known as an endospore. Endospores can be readily recognized microscopically by their intracellular site of formation, their extreme refractility, and their resistance to staining by basic aniline dyes that readily stain vegetative cells. They are not normally formed during active growth and division, their differentiation begins when a population of vegetative cells passes out of the exponential growth phase as a consequence of nutrient limitation.

Differences in the cell wall of Gram positive and Gram negative eubacteria

CHARACTER	GRAM POSITIVE	GRAM NEGATIVE
1. Thickness	-Thicker wall (20 – 25 nm)	-Thinner (10-15 nm)
2 Layers	-A single thick layer	Two layers (a Peptidoglycan layer and outer membrane).
3 Peptidoglycan	Account for 50% dry weight of cell wall	Only about 10% of cell wall.
4 Other constituents	Polysaccharides and Techoic acids	Outer membrane is rich in phospholipids, proteins or lip polysaccharides. Peptidoglycan layer is linked to outer –membrane by Braun's lipoprotein.
5. Susceptibility to a) Penicillin b) Mechanical disintegration	More susceptible Less susceptible	Less susceptible More susceptible

STAPHYLOCCOUS

Staphylococci are gram positive cocci that occur in groups in cluster. They are ubiquitous and most common cause of localized lesions in human beings. They develop resistance to penicillin and other antibiotics.

Staphylococci was first observed in human by Von Recklinghausen. Sir Alexander Oysten established the causative role of coccus in abscesses and other lesions. He named in staphylococcus which means, staphylo – bunches of grapes, kokkos means a berry because of the grape like clusters

in cultures. Staphylococcae strains from pyogenic lesions produce yellow colonies and white colonies from normal skin.

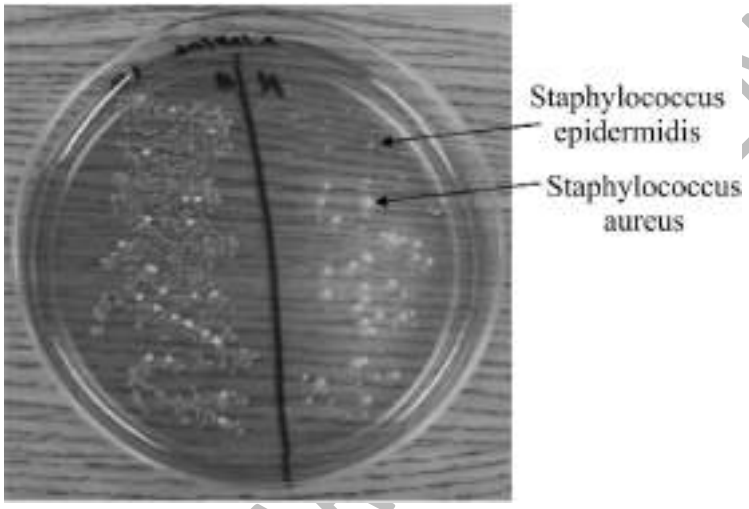
Classification

- Staphylococcus aureus – gives positive coagulase-test, ferments mannitol and mostly pathogenic
- Staphylococcus epidermidis contains coagulase negative non ferments with mannitol and mostly non-pathogenic

STAPHYLOCOCCUS AUREUS

Morphology

They are spherical in shape which are approximately 1µm in diameter arranged in grape like clusters. These are non-motile and non-spring. They are uniformly Gram Positive



Cultural characteristics

They grow readily on ordinary media with temperature ranging from 10-42°C, optimum being 37°C with pH of 7.4 – 7.6 and they are aerobes.

On nutrient agar, the colonies are large (2-4 diameter) circular, convex, smooth, opaque and easily emulsifiable. Most strains produce pigment optimally at 22°C and in aerobic cultures which is enhanced by adding 1% glycerol monacetate or milk in the medium. Colonies on blood agar are similar to that of nutrient agent.

Several selective media containing (8-10% NaCl) like salt-milk agar, salt broth, Lithium chloride and tellurite helps in isolating S.aeures from specimen of Faeces.

Biochemical reactions

They ferment many sugars producing acid but not gases. S.aeures ferments mannitol mostly. They are Catalyst positive, reduces nitrates to nitrites

Characteristics

- Coagulase positive
- Greater biochemical activity, ferment mannitol
- Produce clear haemolysis on blood agar
- Produce a golden yellow pigment
- Liquefy gelatine
- Produce phosphatise

Resistance

They are more resistant nonsporing bacteria. They retain their viability for 3-6 months. Staphylococci may withstand 60ºc for minutes, with thermal death point of 62ºC for 30 minutes. Heat resistant strains may grow even at high temperatures as 45ºC. Most strains grow in the presence of 10% NaCl and some even in 15% NaCl.

Staphylococci were uniformly sensitive to penicillin and some strains produce pencillinase. Pencillinase resistant are of three types namely

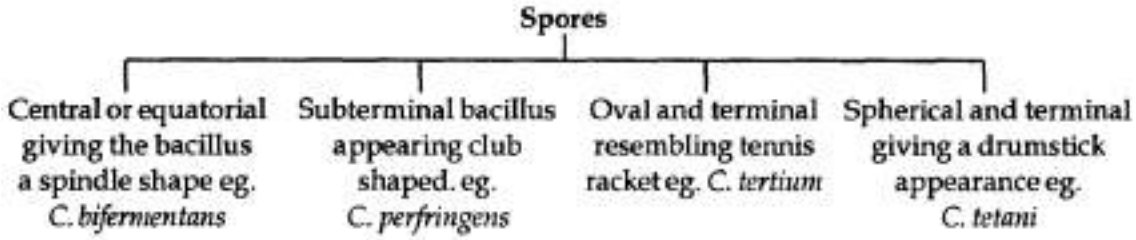
- Produce beta lactamase (pencillinase) which inactivates penicillin by splitting the beta lactam ring. Staphylococci produce four types of pencilinases A to D & hospital stains are usually type A pencillinase
- Changes the bacterial surface receptors reducing binding of beta lactam antibiotics to cells. This also covers beta lactamase resistant pencillin such as Methicillin and Cloxacillin. They are called Methicillin Resistant Staphylococcus Aureus (MRSA). As methicillin is a unstable drug cloxacillin is used for sensitivity testing
- Development of tolerance to penicillin, by which the bacterium is only inhibited but not killed
- Staphylococci shows resistances to all clinical useful antibiotics like erythromycin, tetracycline, and aminoglycosides and hence vancomycin is found useful.

CLOSTRIDIUM

Consists of Gram + ve, anaerobic, spore forming bacilli. The spores are wider than bacillary bodies giving the bacillus a swollen appearance resembling a spindle. Hence the name Clostridium (kloster = spindle). The genus is responsible for three major diseases of human beings-gas gangrene, food poisoning, tetanus.

Many species are pathogenic but most are saprophytic found in soil, water and decomposing plants and animals. Some C. acetobutylicum are used for the production of acetone and butanol.

- They are highly pleomorphic, rod shaped 3-8 f..lm x 0.4-1.2 f..lm long filaments and involution are common. Some sporulate readily while other do rarely. The shape and position of spores are used for identification and classification of clostridia.



- They are motile with peritrichous flagella.
- Gram + ve but older stain is gram variable (gram-ve)
- Anaerobic optimum temperature is 37°C (some thermophilic others psychrophylic) optimum pH = 7–7.4
- Pathogenic *Clostridia* form powerful exotoxins. *C. botulinum* causing botulism is due to ingestion of performed toxin in food. *C. tetani* causing tetanus results from the action of potent exotoxin it produces.

C. tetani: The causative organism of tetanus has been known from very early times being described by Hippocrates and Aretaeus. Rosenbach (1886) demonstrated a slender bacillus with round terminal spores in case of tetanus. The final proof of etiological role of bacillus was furnished by Kitasato (1889).

It is widely distributed in soil and intestine of human beings and animals.

- Obligate anaerobe
- It produces at least two distinct toxin

a) Haemolysin (tetanolysin)-heat 02 labile
b) Neurotoxin (tetanospasmin)-responsible for tetanus

Incubation period is 6-12 days. It is a very serious disease with a high rate of mortality (80-90%). It is very common in developing countries where climate is warm and rural areas where soil is fertile

and highly cultivated (where human and animal population lives in close association and unhygienic practices are common).

- Prevention is by active immunization.

C. botulinum was 1st isolated by Van Frmengem (1896)

- 8 types of C. botulinum have been identified (Type A,B,C1,C2,D,E,F,G) based on immunological difference in the toxins produced by them).
- They produces powerful exotoxin responsible for pathogenicity and it is not released during the life of the organism, produced intra cellularly and appears in the medium only on the death and autolysis of the cell.
- **C. difficle** is not found to be responsible for antibiotic associated colitis by the production of an enterotoxin as well as cytotoxin.

NISSERIAE

The Neisseria are Gram-negative cocci that usually occur in pairs. They are aerobic, nonsporulating, nonmotile, oxidase-positive cocci typically arranged in pairs. N.meningitidis and N. gonorrheae are medically important pathogens, and are found associated with or inside polymorphonuclear cells. Some Neisseria sp are normal inhabitants of the human respiratory tract.

NEISSERIA MENINGITIDIS

Morphology

Meningococci are Gram-negative, oval or spherical cocci, 0.6 – 0.8 µm in size, typically arranged in pairs, with the adjacent sides flattened.

Cultural characteristics

Meningococci have exacting growth requirements and do not grow on ordinary media. Growth occurs on media enriched with blood, serum or ascetic fluid, which promote growth by neutralizing certain inhibiting substances in culture media rather than by providing additional nutritional needs.

They are strict aerobes, no growth occurs anaerobically. The optimum temperature for growth is 35-36oC. No growth takes place below 30oC. Optimum pH is 7.4-7.6. Growth is facilitated by 5-10 percent CO2 and high humidity. On solid media after incubation for 24 hrs, the colonies are small translucent, round, convex, bluish grey, with a smooth glistering surface and with entire edges. Blood agar, chocolate agar and Mueller-Hinton starch casein hydrolysate agar are the media commonly used for culturing meningococci.

Biochemical reactions

They are catalase and oxidase positive, the prompt oxidase reaction helps in the identification of Neisseria in mixed cultures. When a freshly prepared 1% solution of oxidase reagent is poured on the culture media, the Neisseria colonies turn deep purple. Indole and hydrogen sulphide are not produced and nitrates are not reduced. Glucose and maltose are utilized, but not sucrose or lactose, producing acid but no gas.

Antigenic properties and classification

Meningococci are capsulated, unlike other Neisseria. Based on their capsular polysaccharide antigens, meningococci are classified into at least 13 serogroups, of which Groups A,B and C are most important. Group A is usually associated with epidemics and Group C mostly with localized outbreaks, while Group B caused both epidemics and outbreaks.

Resistance

Meningococci are very delicate organisms being highly susceptible to heat, desiccation, alterations in pH and to disinfectants. They are sensitive to penicillin and other antibiotics, but resistance strains have emerged and become common in many areas.

Pathogenicity

Cerebrospinal meningitis and meningococcal septicaemia are the two main types of meningococcal disease. Meningococci are strict human parasites inhabiting the nasopharynx. Infection is usually asymptomatic. In some, local inflammation ensues, with rhinitis and pharyngitis. Dissemination occurs only in a small proportion. Most common complication include Waterhouse-Frederickson syndrome, a massive, usually bilateral haemorrhage into the adrenal glands caused by fulminant meningococcemia, adrenal insufficiency and disseminated intravascular coagulation.

Laboratory diagnosis

In meningococcal meningitis, the cocci are present in large numbers in the spinal fluid and, in the early stage in the blood as well. Demonstration of meningococci in the nasopharynx helps in the detection of carriers.

(a) Examination of CSF

The fluid will be under pressure and turbid, with a large number of pus cells. For bacteriological examination, if a sufficient quantity is available, the CSF is divided into three portions. One portion is centrifuged and Gram- stained smears are prepared from the deposit. Meningococci will be seen mainly inside polymorphs but often extracellularly also. The second portion of the CSF is inoculated in blood agar or chocolate agar plates and incubated at 35-36oC under 5-10% CO2. Colonies appear after 18-24 hrs which may be identified by morphological and biochemical reactions. The third portion of the CSF is incubated overnight either as it is or after adding an equal volume of glucose broth and then subculture

on chocolate agar.

(b) Blood culture

Meningococcemia and in early cases of meningitis, blood culture is often positive. Cultures should be incubated for 4-7 days, with daily subcultures.

(c) Nasopharyngeal swab

This is useful for the detection of carriers. The swab should be held in a suitable transport medium like stuart's medium

(d) Petechial lesions

Meningococci may sometimes be demonstrated in petechial lesions by microscopy and culture.

(e) Molecular diagnosis

Group-specific diagnosis of infection can be made by detection of meningococcal DNA sequence in CSF or blood by PCR amplifications.

Treatment

Prompt treatment is essential to ensure recovery without sequelae. Intravenous penicillin G is the treatment of choice. Chloramphenicol is equally effective.

ESCHERICHIA COLI

Escherichia coli (commonly abbreviated ***E. coli***) is a Gram-negative, facultative anaerobic, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in humans, and are occasionally responsible for product recalls due to food contamination. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K, and by preventing the establishment of pathogenic bacteria within the intestine.

E. coli and related bacteria constitute gut flora, and fecal – oral transmission is the major route through which pathogenic strains of the bacterium cause disease. Cells are able to survive outside the body for a limited amount of time, which makes them ideal indicator organisms to test environmental samples for fecal contamination.

The bacterium can be grown easily and inexpensively in a laboratory setting, and has been intensively investigated for over 60 years. *E. coli* is the most widely studied prokaryotic model organism, and an important species in the fields of biotechnology and microbiology, where it has served as the host organism for the majority of work with recombinant DNA.

SCIENTIFIC CLASSIFICATION

Domain: Bacteria
Kingdom: Eubacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Enterobacteriales
Family: Enterobacteriaceae
Genus: *Escherichia*

HABITAT

Escherichia coli are common inhabitants of the terminal small intestine and large intestine of mammals. They are often the most abundant facultative anaerobes in this environment. They can occasionally be isolated in association with the intestinal tract of nonmammalian animals and insects. The presence of E. coli in the environment is usually considered to reflect fecal contamination and not the ability to replicate freely outside the intestine. There is evidence however to suggest that E. coli may freely replicate in tropical fresh water (Bermudez and Hazen, 1988).

MORPHOLOGY

E. coli is Gram-negative, facultative anaerobic and non-sporulating. Cells are typically rod-shaped, and are about 2.0 micrometers (µm) long and 0.25-1.0 µm in diameter, with a cell volume of 0.6–0.7 µm. It can live on a wide variety of substrates.

Strains that possess flagella are motile. The flagella have a peritrichous arrangement.

CULTURAL CHARACTERISTICS

Escherichia coli or *E.coli* cells may grow on a solid or in a liquid growth medium under a laboratory condition. Solid and liquid media may have exactly the same composition except that the solid medium contains an extra 1.5% agar. Different *E.coli* clones may have different properties. Colonies growing on solid media represent different clones.

BIOCHEMICAL REACTIONS

E. coli uses mixed-acid fermentation in anaerobic conditions, producing lactate, succinate, ethanol, acetate and carbon dioxide. Since many pathways in mixed acid fermentation produce hydrogen gas, these pathways require the levels of hydrogen to be low, as is the case when *E. coli* lives together with hydrogen consuming organisms, such as methanogens or sulphate-reducing bacteria. Optimal growth of *E. coli* occurs at 37°C (98.6°F) but some laboratory strains can multiply at temperatures of up to 49°C (120°F).

ROLE IN DISEASES

The commonest infection caused by *E. coli* is infection of the urinary tract, the organism normally spreading from the gut to the urinary tract. *E. coli* is also the commonest cause of cystitis (infection of the bladder), and in a minority of patients the infection may spread up the urinary tract to the kidneys, causing pyelonephritis. Otherwise healthy patients in the community may develop

cystitis, and patients in hospital who have catheters, or tubes, placed in the urethra and bladder are also at risk. *E. coli* is also present in the bacteria that cause intra-abdominal infections following leakage from the gut into the abdomen, as for example with a ruptured appendix or following traumatic injury to the abdomen.

E. coli bacteria may also cause infections in the intestine. Diarrhoeal infections (intestinal) are caused by a group of *E. coli* known as 'enter virulent' (harmful to the intestines).

Overspill from the primary infection sites to the bloodstream may cause blood poisoning (*E. coli* bacteraemia). In rare instances, *E. coli* may cause meningitis in very young children.

FUNGI

Fungi can be single celled or very complex multicellular organisms. They are found in just about any habitat but most live on the land, mainly in soil or on plant material rather than in sea or fresh water. A group called the decomposers grow in the soil or on dead plant matter where they play an important role in the cycling of carbon and other elements. Some are parasites of plants causing diseases such as mildews, rusts, scabs or canker. In crops fungal diseases can lead to significant monetary loss for the farmer. A very small number of fungi cause diseases in animals. In humans these include skin diseases such as athletes' foot, ringworm and thrush.

Types of fungi

Fungi are subdivided on the basis of their life cycles, the presence or structure of their fruiting body and the arrangement of and type of spores (reproductive or distributional cells) they produce.

The three major groups of fungi are:

- Multicellular filamentous moulds.
- Macroscopic filamentous fungi that form large fruiting bodies. Sometimes the group is referred to as 'mushrooms', but the mushroom is just the part of the fungus we see above ground which is also known as the fruiting body.
- Single celled microscopic yeasts

Multicellular filamentous moulds

Moulds are made up of very fine threads (hyphae). Hyphae grow at the tip and divide repeatedly along their length creating long and branching chains. The hyphae keep growing and intertwining until they form a network of threads called a mycelium. Digestive enzymes are secreted from the hyphal tip. These enzymes break down the organic matter found in the soil into smaller molecules which are used by the fungus as food.

Some of the hyphal branches grow into the air and spores form on these aerial branches. Spores are specialised structures with a protective coat that shields them from harsh environmental conditions such as drying out and high temperatures. They are so small that between 500 – 1000 could fit on a pin head.

Spores are similar to seeds as they enable the fungus to reproduce. Wind, rain or insects spread spores. They eventually land in new habitats and if conditions are right, they start to grow and produce new hyphae. As fungi can't move they use spores to find a new environment where there are fewer competing organisms.

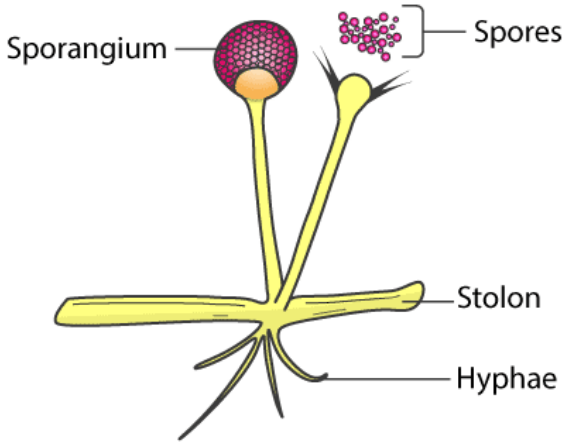
Macroscopic filamentous fungi

Macroscopic filamentous fungi also grow by producing a mycelium below ground. They differ from moulds because they produce visible fruiting bodies (commonly known as mushrooms or toadstools) that hold the spores. The fruiting body is made up of tightly packed hyphae which divide to produce the different parts of the fungal structure, for example the cap and the stem. Gills underneath the cap are covered with spores and a 10 cm diameter cap can produce up to 100 million spores per hour.

Yeasts

Yeasts are small, lemon-shaped single cells that are about the same size as red blood cells. They multiply by budding a daughter cell off from the original parent cell. Scars can be seen on the surface of the yeast cell where buds have broken off. Yeasts such as Saccharomyces play an important role in the production of bread and in brewing. Yeasts are also one of the most widely used model organisms for genetic studies, for example in cancer research. Other species of yeast such as Candida are opportunistic pathogens and cause infections in individuals who do not have a healthy immune system.

Structure of Fungi



The structure of fungi can be explained in the following points:

1. Almost all the fungi have a filamentous structure except the yeast cells.
2. They can be either single-celled or multicellular organisms.
3. Fungi consist of long thread-like structures known as hyphae. These hyphae together form a mesh-like structure called mycelium.
4. Fungi possess a cell wall which is made up of chitin and polysaccharides.
5. The cell wall comprises a protoplast, which is differentiated into other cell parts such as cell membrane, cytoplasm, cell organelles and nuclei.
6. The nucleus is dense, clear, with chromatin threads. The nucleus is surrounded by a nuclear membrane.

Characteristics of Fungi

Following are the important characteristics of fungi:

1. Fungi are eukaryotic, non-vascular, non-motile and heterotrophic organisms.
2. They may be unicellular or filamentous.
3. They reproduce by means of spores.
4. Fungi exhibit the phenomenon of alternation of generation.
5. Fungi lack chlorophyll and hence cannot perform photosynthesis.
6. Fungi store their food in the form of starch.
7. Biosynthesis of chitin occurs in fungi.
8. The nuclei of the fungi are very small.
9. The fungi have no embryonic stage. They develop from the spores.
10. The mode of reproduction is sexual or asexual.
11. Some fungi are parasitic and can infect the host.
12. Fungi produce a chemical called pheromone which leads to sexual reproduction in fungi.
13. Examples include mushrooms, moulds and yeast.

Classification of Fungi

Kingdom Fungi are classified based on different modes. The different classification of fungi is as follows:

Based on Mode of nutrition

On the basis of nutrition, kingdom fungi can be classified into 3 groups.

1. **Saprophytic** – The fungi obtain their nutrition by feeding on dead organic substances. Examples: Rhizopus, Penicillium and Aspergillus.

2. **Parasitic** – The fungi obtain their nutrition by living on other living organisms (plants or animals) and absorb nutrients from their host. Examples: Taphrina and Puccinia.

3. **Symbiotic** – These fungi live by having an interdependent relationship with other species in which both are mutually benefited. Examples: Lichens and mycorrhiza. Lichens are the symbiotic association between algae and fungi. Here both algae and fungi are mutually benefited as fungi provide shelter for algae and in reverse algae synthesis carbohydrates for fungi. Mycorrhiza is the symbiotic association present between fungi and plants. Fungi improve nutrient uptake by plants, whereas, plants provides organic molecules like sugar to the fungus.

Based on Spore Formation

Kingdom Fungi are classified into the following based on the formation of spores:

1. **Zygomycetes** – These are formed by the fusion of two different cells. The sexual spores are known as zygospores, while the asexual spores are known as sporangiospores. The hyphae are without the septa. Example – Mucor.

2. **Ascomycetes** – They are also called sac fungi. They can be coprophilous, decomposers, parasitic or saprophytic. The sexual spores are called ascospores. Asexual reproduction occurs by conidiospores. Example – Saccharomyces.

3. **Basidiomycetes** – Mushrooms are the most commonly found basidiomycetes and mostly live as parasites. Sexual reproduction occurs by basidiospores. Asexual reproduction occurs by conidia, budding or fragmentation. Example- Agaricus.

4. **Deuteromycetes** – They are otherwise called imperfect fungi as they do not follow the regular reproduction cycle as the other fungi. They do not reproduce sexually. Asexual reproduction occurs by conidia. Example – Trichoderma.

Aspergillus

Aspergillus (Plural Aspergilli) is a genus of fungi that consists of about 300 identified species of mold (mould). Aspergillus can be found in a variety of environments throughout the world given that their growth is largely determined by the availability of water.

Kingdom: **Fungi**
Phylum: **Ascomycota**
Order: **Eurotiales**
Family: **Trichocomaceae**
Genus: **Aspergillus**

A majority have been shown to be terricolous, which means that they are largely found in soil/land. Since their discovery in the 1720s, they have become increasingly important in human health, agriculture as well as in biological sciences among others.

General Characteristics of Aspergillus

Aspergilli can be found throughout nature with their spores being abundant in air. In addition to largely being saprophytes that obtain their nutrition from dead and decaying matter, they can also be pathogenic to human beings and animals with some also affecting and damaging plants.

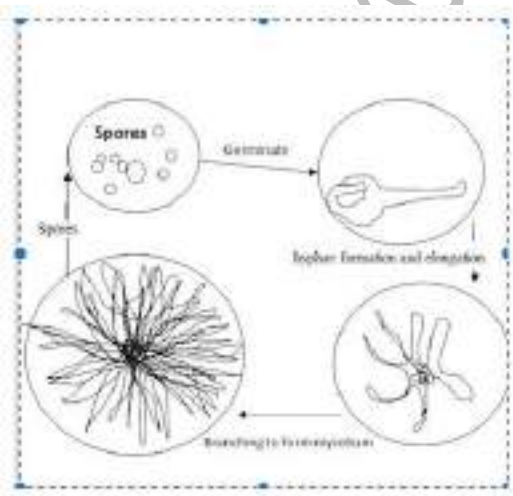
Because they lack chlorophyll, and are therefore unable to produce their own food, they are dependent on other material in their surroundings for nutrition. Here, it's worth noting that Aspergilli are incapable of absorbing organic matter in their surroundings.

For this reason, they release different types of enzymes such as amylase capable of breaking down these materials into simpler compounds that can be absorbed through the vegetative hyphae.

The release of high amounts of these enzymes results in enhanced decay of all organic matter in their immediate environment and consequently the availability of more food source necessary for reproduction and growth.

For most part, they reproduce asexually through the production of spores known as conidium (fungi spore). Once the spore lands on a favourable environment (with moisture, warmth and nutrients) they start germinating where they create numerous hyphae that form the mycelium.

The hyphae allows them to grow, spread and continue reproducing across the surface of the substrate.



Aspergilli's hyphae develops into vegetative and reproductive hyphae. The vegetative hyphae serves to absorb nutrients while the reproductive hyphae develop further to produce spores.

Algae

Algae exist in environments ranging from oceans, rivers, and lakes to ponds, brackish waters and even snow. Algae are usually green, but they can be found in a variety of different colours. For instance, algae living in snow contain carotenoid pigments in addition to chlorophyll, hence giving the surrounding snow a distinctive red hue.

Multicellular examples of algae include the giant kelp and brown algae. Unicellular examples include diatoms, Euglenophyta and Dinoflagellates.

Most algae require a moist or watery environment; hence, they are ubiquitous near or inside water bodies. Anatomically, they are similar to another major group of photosynthetic organisms – the land plants. However, that is where the differences end as algae lack many structural components typically present in plants, such as true stems, shoots, and leaves. Furthermore, they also do not have vascular tissues to circulate essential nutrients and water throughout their body.

Characteristics of Algae

Specific general characteristics of algae are common to plants as well as animals.

For instance, algae can photosynthesize like plants, and they possess specialized structures and cell-organelles, like centrioles and flagella, found only in animals. Listed below are some of the general characteristics of algae.

- Algae are photosynthetic organisms
- Algae can be either unicellular or multicellular organisms
- Algae lack a well-defined body, so, structures like roots, stems or leaves are absent
- Algae are found where there is adequate moisture.
- Reproduction in algae occurs in both asexual and sexual forms. Asexual reproduction occurs by spore formation.
- Algae are free-living, although some can form a symbiotic relationship with other organisms.

Types of Algae

There are many types of algae. However, these are some of the more prominent types:

Red Algae

Also called Rhodophyta, it is a distinctive species found in marine as well as freshwater ecosystems. The pigments phycocyanin and phycoerythrin are responsible for the characteristic red colouration of the algae. Other pigments that provide green colouration (such as chlorophyll a) are present. However, they lack chlorophyll b or beta-carotene.

Green Algae

It is a large, informal grouping of algae having the primary photosynthetic pigments chlorophyll a and b, along with auxiliary pigments such as xanthophylls and beta carotene.

Higher organisms use green algae to conduct photosynthesis for them. Other species of green algae have a symbiotic relationship with other organisms.

Members are unicellular, multicellular, and colonial and flagellates. Prominent examples of green algae include Spirogyra, Ulothrix, Volvox, etc.

Ultrastructure of Eukaryotic Algal Cell:

Chlamydomonas, a member of green algae (chlorophyceae) is found almost in all places. It is simple, motile, unicellular, fresh water alga. Its ultrastructure can be divided into following parts

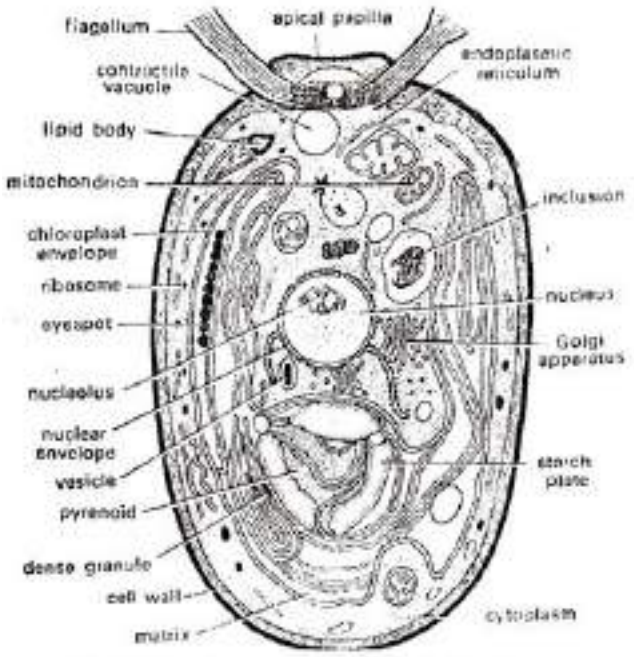


Fig. 1. *Chlamydomonas*. Ultrastructure of eukaryotic cell.

Cell Wall of Eukaryotic Algal Cell:

The cell is bounded by a thin, cellulose cell wall. Cellulose layer is finely striated with parallel cellulose fibrils (Fig. 1). In many species there is a pectose layer external to it which dissolves in water and forms a mucilaginous pectin layer. According to Roberts et. al. (1972), Hills (1973) the cell wall in C. Reinhardt consists of seven layers.

Plasma Lemma of Eukaryotic Algal Cell:

It is present just below the cell wall and consists of two opaque layers which remain separated by less opaque zone (Fig. 1).

Protoplast of Eukaryotic Algal Cell:

It is bounded by plasma lemma. It is differentiated into cytoplasm, nucleus, and chloroplast with one or more pyrenoids, mitochondria, Golgi bodies, two contractile vacuoles, a red eye spot and two flagella.

Chloroplast of Eukaryotic Algal Cell:

In majority of the species of Chlamydomonas, cytoplasm contains of a single, massive cup shaped chloroplast which almost fills the oral or pear shaped body of the cell. It is surrounded by a double-layered unit membrane. It bears number of photosynthetic lamellae (disc or thylakoids).

The lamellae are lippo-proteinaceous in nature and remain dispersed in a homogeneous granular matrix (stroma). About 3-7 thylakoids bodies fuse to form grana like bodies. Matrix also contains ribosomes, plastoglobuli, microtubules and many crystals like bodies.

Flagella of Eukaryotic Algal Cell:

The anterior part of thallus bears two flagella. Both the flagella are whiplash or acronematic type, equal in size. Each flagellum originates from a basal granule or blepharoplasty and comes out through a fine canal in cell wall. It shows a typical 9+ 2 arrangement. Fibrils remain surrounded by a peripheral fibril. According to Ringo (1907), 2 central ones are singlet fibrils and 9 peripheral ones are doublet fibrils (Fig. 2).

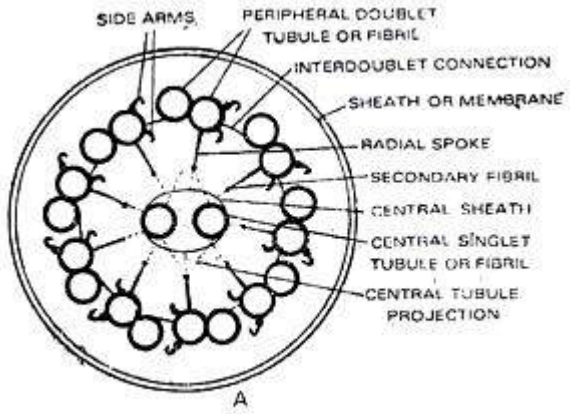


Fig. 2. Ultrastructure of flagellum of *Chlamydomonas.*

Stigma or Eyespot of Eukaryotic Algal Cell:

The anterior side of the chloroplast contains a tiny spot of orange or reddish colour called stigma or eyespot. It is photoreceptive organ concerned with the direction of the movement of flagella. The eye spot is made of curved pigmented plate. The plate contains 2-3 parallel rows of droplets or granules containing carotenoids (Fig. 3).

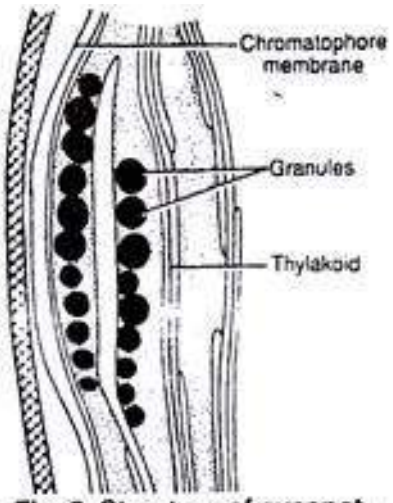


Fig. 3. Structure of eyespot.

The other structures such as mitochondria, Golgi bodies, endoplasmic reticulum and nucleus are also bounded by double-layered unit membrane.

Occurrence of Chlamydomonas:

The genus Chlamydomonas (Gr. Chlamys, mantle; Monas, single organism) includes about 500 species, found almost everywhere (i.e., ubiquitous). Commonly they are found in fresh water of lakes, ponds, tanks etc., but they are also available in brackish water (C. halophila), saline water (C. ehrenbergii), snow (C. nivalis) and some are also air borne.

The snow of arctic and alpine zones becomes red due to the presence of C. nivalis which accumulates the red pigment haematochrome. But the snow covered mountain range of yellow

stone national park (USA) becomes yellowish green due to the well- developed population of C. yellowstonensis.

Plant Body of of Chlamydomonas:

Plant body of Chlamydomonas is unicellular and motile (Fig. 3.41). The cells are usually spherical, oval or oblong in shape but other forms like ellipsoidal, pyriform etc. are also available (Fig. 3.39). Most of the species are broader towards the posterior side and pointed towards the anterior side, which gradually ends in apical papilla.

The length of the cell varies from 20 to 30µm but rarely exceeds 30µm in major diameter.

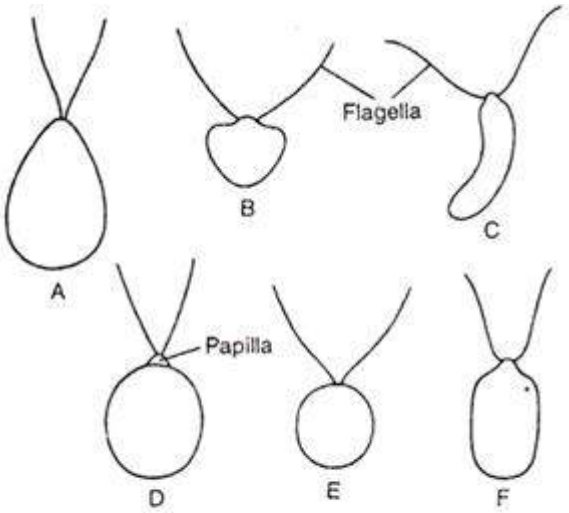


Fig.3.39 . *Chlamydomonas* : Plant body of various shapes. A. *C. polydactyla*, B. *C. gyroides*, C. *C. lunata*, D. *C. conosa*, E. *C. incerta*, and F. *C. biconvexa*

The cell wall is thin, smooth and firm. It is made up of cellulose. The major structural component of cell wall is glycoprotein. In some species (C. gleocystiformis), the cell wall is surrounded by mucilaginous pectin layer formed by the dissolution of pectose layer to pectin.

Inner to the cell wall, semipermeable cell membrane or plasma membrane is present which surrounds the protoplast.

The protoplast contains the following:

a. Chloroplasts:

It occupies the lower broader part. It is generally of cup-shaped and parietal (Fig. 3.40). (However, the chloroplast is variable in shape, such as H-shaped in C. biciliata; parietal in C. mucicola; reticulate in C. reticulata and stellate in C. arachne).

Chloroplast has single pyrenoid with a starch sheath. The number is also variable and it may be two (C. debaryana) to many (C. gigantae). Sometimes they may be numerous and distributed irregularly inside the chloroplast (C. sphgnicola).

b. Eye-Spot:

Towards the anterior end of the chloroplast at one side an circular to oval, photoreceptive organ, the stigma or eye spot is present. The spot consists of a curved pigmented plate,, the pigmentosa and a biconvex lens.

c. Nucleus:

The nucleus remains suspended inside the cups and is of prokaryotic in nature.

d. Other Inclusion:

It includes mitochondria, E.R. vesicles, contractile vacuoles etc.

e. Flagella:

Two whiplash-types of flagella are present towards the anterior region of the cell. They are equal in length. The flagella may be very small, same size of the cell or bigger than the cell in most species.

The flagellum originates from the blepharoplasty, situated towards the anterior side (Fig. 3.42). The flagella come out through very fine canals, on the outer wall.

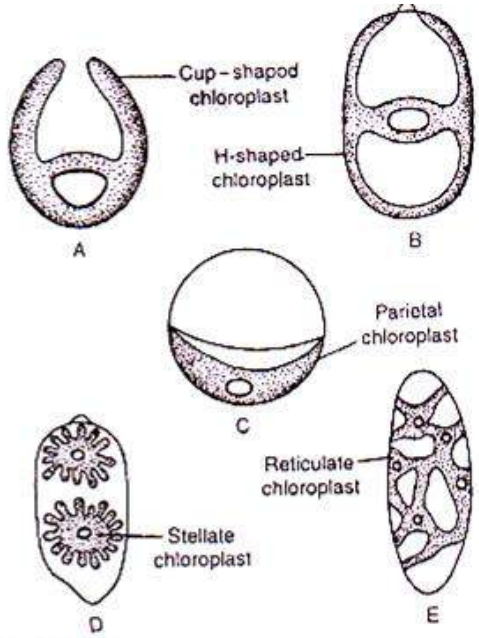


Fig 3.40 : *Chlamydomonas* : Types of chloroplasts. A. Cup-shaped (*C. eugametos*), B. H-shaped (*C. bicillata*), C. Parietal (*C. mucicola*), D. Stellate (*C. arachne*) and E. Reticulate (*C. reticulata*)

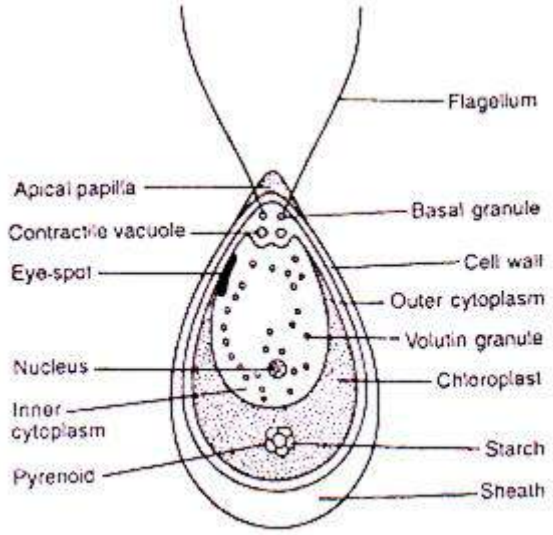


Fig. 3.41 : *Chlamydomonas* : A vegetative cell

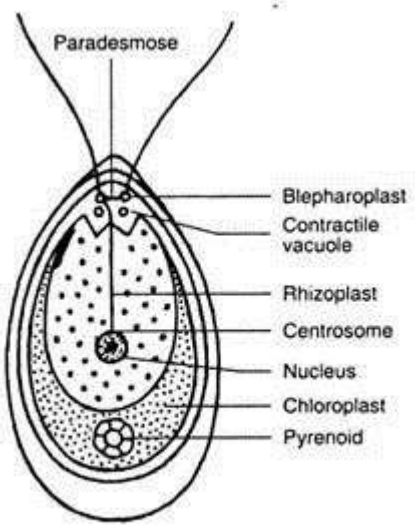


Fig. 3.42 : *Chlamydomonas* showing neuromotor apparatus

In some species like C. nasuta neuromotor apparatus (Fig. 3.42) is said to be present. The neuromotor apparatus consists of two basal granules, the blepharoplasts. Both are connected by a fibre, called paradesmose. One of the blepharoplast is connected to the centrosome of the nucleus by a thread, the rhizoplast. Many fine fibrils connect the centrosome with the nucleolus.

f. Contractile Vacuoles:

Two contractile vacuoles are present just below the blepharoplast. Possibly they regulate the water content of the cell, by discharging more water at times.

Important Features of Chlamydomonas:

1. Plant body is unicellular, pear-shaped and biflagellate.
2. Each cell has generally cup-shaped chloroplast, one eye-spot and two contractile vacuoles.
3. Presence of palmella-stage.
4. Asexual reproduction takes place through biflagellate zoospore formation.
5. Sexual reproduction through iso-, aniso-, and oogamy.

AMOEBAE

Amoebae can be pathogenic called Entamoeba histolytica and non-pathogenic called Entamoeba coli (large intestines), Entamoeba gingivalis (oral cavity). These parasites are motile with pseudopodia. The pseudopodia are cytoplasmic processes which are thrown out.

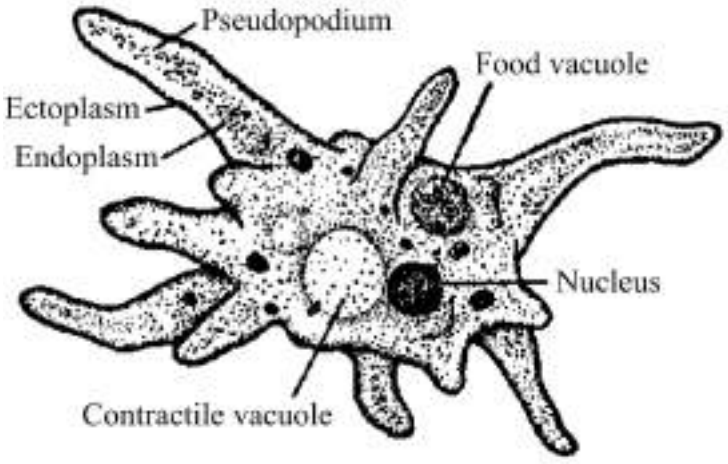
ENTAMOEBA HISTOLYTICA

It belongs to the class Rhizopoda and family Entamoebidae. It is the causative agent of amoebiasis. Amoebiasis can be intestinal and extra intestinal like amoebic hepatitis, amoebic liver abscess.

MORPHOLOGY

The Entamoeba is seen in three stages

(a) Trophozoite: The trophozoite is 18-40 µm in size. The trophozoite is actively motile. The cytoplasm is demarcated into endoplasm and ectoplasm. Ingested food particles and red blood cells are seen in the cytoplasm No bacteria are seen in the cytoplasm. The nucleus is 6-15 µm and has a central rounded karyosome. Nuclear membrane has chromatin granules and spoke like radial arrangement of chromatin fibrils.



(b) Precyst: Smaller in size. 10-20 µm in diameter. It is round to oval in shape with blunt pseudopodium. The nuclei is similar to the trophozoite.

(c) Cyst: These are round 10-15 µm in diameter. It is surrounded by a refractile membrane called as the cyst wall. The cyst wall makes it resistant to gastric juices. The nuclei are similar to the trophozoite. Mature cyst has four nuclei. The nuclei initially divides into two and then to four by binary fission. The uninucleate and binucleate stage also has a glycogen mass. Cysts are seen only in the lumen of the colon and in the stools.

LIFE CYCLE OF ENTAMOEBA HISTOLYTICA

The life cycle is spent in only one host i.e. man. The mature quadrinucleate cysts are the infective forms. The cysts are ingested in food and water and reach the ceacum or the lower part of ileum where the excystation of the cyst occurs. The mature cyst liberates a single amoeba with four nuclei (Tetranucleate amoeba).

The nuclei further divide to produce the eight metacystic trophozoites. These trophozoites lodge in the submucosa of large intestine. In the large intestines they grow and multiply by binary fission.

PATHOGENECITY

After an incubation period ranging from 4-5 days to 3-4 weeks the disease starts presenting as loose diarrhoea which may have blood and stool mixed in it. E. histolytica secretes a proteolytic enzyme which causes destruction and necrosis of intestinal mucosal tissue leading to formation of flask shaped ulcers. A large number of trophozoites and cysts mixed with blood and mucus are excreted in the feaces presenting as dysentery which is referred to as amoebic dysentry.

Some trophozoites gain entry into the portal vein and reach the liver. In the liver it can cause. The pus in the liver abscess is reddish brown in colour and is like anchovy sauce.

(a) Amoebic hepatitis

(b) Amoebic liver abscess.

The trophozoites transform to precyst and cysts and cysts are excreted in feaces. The mature cyst is the infective form of E histolytica.

VIRUSES

Viruses may be generalized to define as 'very small sized etiological agents of disease that are capable of passing through filters that retain even bacteria, increase only in the presence of living cells, and give rise to new strains by mutation'. Mayer (1886) showed that the juice from the infected plants of tobacco could reproduce the disease if applied to healthy plants. The Russian botanist Dimitri Ivanowski (1892), demonstrated that the causal organism of tobacco mosaic could even pass through the finest porcelain filter that withholds bacteria. Ivanowski also showed that this filterate was capable of transmitting the disease to healthy susceptible plants. He also indicated that these causal organism were even smaller than bacteria.

Beijerinck (1898) a Dutch microbiologist, showed that the causal agent or tobacco mosaic could diffuse through an agar membrane and was therefore liquid in nature such a liquid causal agent of tobacco mosaic was called by Beijerinck as "Contagium vivum fluidium' or 'living infection fluid'.

Bacteriophages (viruses that parasitise bacteria) were discovered by the French scientist D. Herelle (1917) who found that some agent was destroying his cultures of bacilli.

Schelsinger (1933) was the first to determine the decomposition of virus. He showed that a bacteriophage consist of only protein and DNA.

Bowden (1964) defined viruses as 'submicroscopic, infective entities that multiply only intra-cellularly and are potentially pathogenic. According to Hahon (1964) viruses are 'bits of infectious heredity in search of a chromosome'. Some define viruses as 'infectious nucleoproteins'. The word virus is derived from the latin language meaning 'poisonous liquid or 'poison'.

In 1935 Stanley crystallized the virus causing tobacco mosaic disease, and demonstrated that the crystals retained their infectivity when inoculated into healthy plants Hershey and Chase (1952) studied the T2 bacteriophage and demonstrated that (1) the genetic information is carried in the phage DNA and (2) that infection is the result of penetration of viral DNA into cells.

The nucleic acid fraction of the virus is the actual infectious agent was first shown by Gierrer and Schramm (1956).

OCCURRENCE

VIRUSES IN EUKARYOTIC MICROORGANISM

Virus like particles have been observed in species of Protozoa, algae and fungi.

Protozoa: Viruses or virus like particles have been observed in several protozoa viz., leishmania, Entamoeba histolytica, Plasmodium vivax, P. berghei, Paramecium aurelia and Acanthamoeba sp. A virus like particle has been reported in Plasmodium berghei. Its structure is like that of a cytoplasmic Polyhedrosis virus, a dsRNA virus of insects.

Phycophages (parasite of **Algae)** were first isolated by Schafferman and Morris (1963) from blue green alga . The phage isolated by them was found to infect Plectono1lema and Phormidiunl also (hence named LPP-1), these are cyanophages. Some Virus like particles have been reported in Aulacomonas subnzarina, Chara, Corralina, Oedogonium spp., Uronema gigas. Bacteriophage like virus particles have been found in Chlorella and have been called chlorellophages.

Macrophages (parasite of **Fungi)**: They were first discovered in mushroom (Agaricus bisporous) by Sinden in 1957. It affects fungi. Killer phenotypes associated with the strains of Saccharomyces cerervisiae and Ustilago maydis have been shown to be virus related. dsDNA viruses have been detected in cells of Penicillium moulds. dsRNA viruses are widely present in the higher fungi. The bacilli form particles of Agaricus bisporous is the only fungal virus reported that contains ssRNA.

The occurrence of viruses in the cells of bacteria and higher plants and animals is well established.

Plant viruses: Most plant viruses have been found in angiosperm (flowering plants). Relatively few viruses are known in gymnosperm, ferns, fungi or algae. Plant viruses are of great economic importance, since they cause plant diseases in a variety of crops.

Animal viruses: Virus diseases are known in a variety of vertebrates including fish, amphibian, birds and mammals. Important virus diseases of humans include poliomyelitis, small pox, rabies, mumps, measles, yellow fever, influenza and encephalitis.

GENERAL CHARACTERS OF VIRUSES

- They do not occur free in nature but act as obiigate intracellular parasite.
- They are extreme microscopic structure which can only be seen by electron microscope.
- Mainly the size ranges from 100-2000 millimicron.
- They can not be filtered by bacterial filters.
- The genetic material is either DNA or RNA which occurs in the form of single molecule and can be single or double stranded.
- A single virus particle is known as virion which lacks functional autonomy.
- They lack their own enzyme system but interact with the host enzyme system and synthesize new virus particles. Thus they have a .master and slave relationship.
- Outer capsid of virus is proteinaceous, harmless and provide cellular specificity to the virus.
- They are intracellular obligate parasite and can't be cultured on artificial culture media.

- All animal and plant viruses have a narrow host range while others show a broad host range.
- They show replication.
- They are highly infectious and spread disease very quickly.
- They show special kind of pathogenecity i.e. they cause disease at particular temperature. Most of virus become inert at 56-69°C (for 30 minutes)
- They are haploid.
- They are unaffected by antibiotics.
- They show life between 5-9 pH.
- They remain active for a long time when kept in 50% glycerol solution.
- The extract of virus become inert at high pressure and high sound frequency.
- They get precipitated with ethyl alcohol and acetone.
- They can be inerted by treatment with ultraviolet rays, pyridine, urea and hydrogen peroxide.
- They can be crystallized.
- They show response toward temperature, radiation and chemical substances.
- They lack cell wall, nucleus, protoplasm and cell organelles.

How do Virus differ from Bacteria and Mycoplasmas?

Viruses differ from bacteria and mycoplasmas in :

- Not possessing any cellular organization.
- Not growing on inanimate media.
- Not multiply by binary fission.
- Not possessing both DNA and RNA together.
- Not possessing ribosome.
- Not showing any sensitivity to antibiotics.
- Showing sensitivity to interferon.

NATURE OF VIRUSES

The nature of viruses is still not clear, because it is not easy to define them within the accepted framework of living or non-living organisms. Some virologist regard viruses as animate object (when present inside the host cell) whereas other consider them inanimate (when present outside the host cell).

Viruses are living because:

(i) They show growth and multiplication (only inside the host cell).
(ii) They have genetic material i.e. DNA/RNA.
(iii) They can direct protein synthesis (though they use host machinery for it).
(iv) They show mutation.
(v) They can be transmitted from the diseased host to the healthy ones or possess the ability to infect.

(vi) They react to heat, chemicals and radiation and also shows irritability, a character of only living organisms.
(vii) They possess genetic continuity and have definite races/strains.
(viii) Similarity between nucleoproteins of viruses with the protein and nucleic acid of living organisms.

Viruses are non-living because:

(i) They can be crystallized (Stanley, 1935)
(ii) They behave as inert chemicals outside the host cell.
(iii) A cell wall or cell membrane of any type is absent in viruses.
(iv) They do not show functional autonomy.
(v) They do not respire or excrete or they do not show any sign of metabolism except reproduction.
(vi) They lack any energy producing enzyme system.

SIZE AND STRUCTURE OF VIRUSES

The size of viruses is variable. Most viruses are much smaller than bacteria. Their size ranges from 10 nm - 250 nm. The size of viruses is determined by electron microscopy, ultra centrifugation and by filtration through colloid ion membrane of known pore diameter.

The smallest virus is coliphage F2 measuring about 2 nm. The smallest plant virus is satellite tobacco necrosis virus measuring 17nm. The longest known plant virus is citrus tristeza virus-rod shaped measuring 2000 x 12 nm. Foot and mouth virus of cattle is the smallest animal virus measuring about 10 nm. Pox viruses are the largest and most complex animal viruses. Parrot fever virus measuring 400 nm.

Structure of Viruses

The intact virus unit or infectious particle is called the virion. Each virion consists of a nucleic acid core surrounded by a protein coat (capsid) to form the nucleocapsid. The nucleocapsid may be naked or may be surrounded by a loose membranous envelope.

It is composed of a number of subunits called capsomeres. The capsid protects the nucleic acid core against the action of nucleases. Structurally viruses occur in three main shapes viz. spherical or polyhedral, cylindrical or helical and the complex type.

POLYHEDRAL (ICOSAHEDRAL) SYMMETRY

Crick & Watson have shown that the polyhedral capsids can nave three possible types of symmetry viz. Tetrahedral, octahedral and icosahedral.

Icosahedral is the most efficient shape for the packing and bonding of subunits of a near spherical virus. In icosahedral symmetry a large number of intermolecular bonds can be formed in this type of structure and is therefore has low free energy.

An icosahedron is a regular polyhedron with 20 faces formed by equilateral triangles and 12 intersecting points or corners.

Each capsid consist of many capsomeres. Each capsomere is composed of a few monomers which form polygonal rings, each with a central space of up to 40 AO. The monomers are the structural units and are made up of one or more polypeptide chains.

There are two types of capsomeres:

(i) Pentamers or pentagonal capsomere is made up of 5 monomers.

(ii) Hexamers or hexagonal capsomere consist of 6 monomers.

HELICAL SYMMETRY

The helical capsid consist of monomers arranged in a helix around a single rotational axis. The monomers curve into a helix because they are thicker at one end than the other. Helical capsids may be naked (e.g. the tobacco mosaic virus) or surrounded by an envelope (e.g. influenza virus).

Tobacco Mosaic Virus

Virus is rod shaped about 300 nm x 15-18 nm in diameter. X-ray diffraction studies have shown that the virus consists of a protein tube with a lumen of 20 A 0 enclosing a single strand of helically coiled RNA. The tube is made up of a number of identical sub units (monomers) of proteins arranged in a helical manner. Studies by Franklin and her co-workers have shown that there are 49 subunits of protein for three turns of the helix, thus giving a total of 2,130 subunits for the rod. Each subunit has a molecular weight of 17, 500 and consist of single poly chain made up of 158 amino acids.

The RNA is a single stranded molecular coiled into a helix 80Ao in diameter. It follows the pitch of the protein helix. Each tum of RNA helix contains about 49 nucleotides with a pitch of 23°.

COMPLEX SYMMETRY

Complex viruses are divided into two groups:

(a) Those without identifiable capsids.

(b) Those with capsids to which are attached additional structures.

Vaccinia virus is an example of a virus without a definite capsid. The nucleic acid is surrounded by several coats.

RHABDOVIRUS

The family Rhabdoviridae consist of more than 200 viruses of vertebrate, invertebrate and plants. It has two genera. Vesiculovirus and Lyssavirus. The member of genus vesiculovirus causes vesicular stomatitis in horses, cattle, and pigs and only one of the 35 serologically distinct viruses of this genus causes human infection. The genus lyssa virus contain rabies virus and five rabies like viruses: Mokola, Lagosbat, Kotonkan, Obodhiang and Duvenhage viruses. Each of these viruses are capable of causing rabies like disease in domestic animals and humans.

The bite of an infected animal like dog, cat, bat or skunk can transmit the disease to man. Morphology (Rabies name come from the latin word rabidus = mad)

Rabies virus is bullet shaped 180 x 75 nm with one end rounded conical and the other plane or concave. The core of the virion consists of a minus sense 11-12 Kb. Single stranded (-) RNA enclosed in helically wound nucleocapsid. RNA dependent RNA polymerase enzyme (required for initiation of replication of virus) is enclosed within the virion in association with the

ribonucleoprotein core. This ribonucleoproein core is surrounded by viral membrane or matrix protein which may be invaginated at the plane.

The matrix protein is again surrounded by a lipoprotein envelope which carries glycoprotein peplomers (spikes). The spikes do not cover the plane end of the virion.

Symptoms

The symptom in human include severe headache and high fever with alternating stages of excitement and depression. Patients have difficulty in swallowing and slight stimuli incite muscular spasms in the throat and chest. Death may occur due to convulsive seizures. The mortality rate in untreated patients is nearly 100%. The course of disease in humans can be classified into four stages- prodrome, acute encephalitic phase, coma and death. The onset is marked by prodromal symptoms like fever, headache, malaise, fatigue, and anorexia. An early symptom is often a neuritic type of pain or Paresthesia and fasciculation at the site of virus entry. Apprehension anxiety, agitation, irritability, nervousness, insomnia or depression characterise the prodromal phase, last for 2-4 days.

Excessive libido, priapism, and spontaneous ejaculation may occur rarely. The acute neurological phase usually begins with hyperactivity with bouts of bizarre behaviour, agitation or seizures appearing between apparently normal periods.

Some patients progress to paralysis. Death is due to respiratory arrest or other complications.

After the bite of a rabid animal the incubation period is usually between 1-2 months. However it may be as short as 9 days and rarely as long as a year or more. It is shorter in children than in adults and also in person bitten on face or head than bitten on the legs. This is related to the distance the virus has to travel to reach 'the brain. Patients develops difficulty in drinking, together with intense thirst. Attempts to drink bring on painful spasm of pharynx and larynx producing choking and gagging. Therefore mere sight or sound of water precipitates distressing muscular spasm leading to hydrophobia (fear of water).

The furious form of rabies, gradually subsides into delirium, convulsions coma and death. Sometimes only the dumb form is seen, with symmetrical ascending paralysis followed by coma and death. The disease, once developed is almost always fatal in 4-14 days. Rabies virus may be secreted in the saliva, urine and other secretions of human rabies victims as in that of animals.

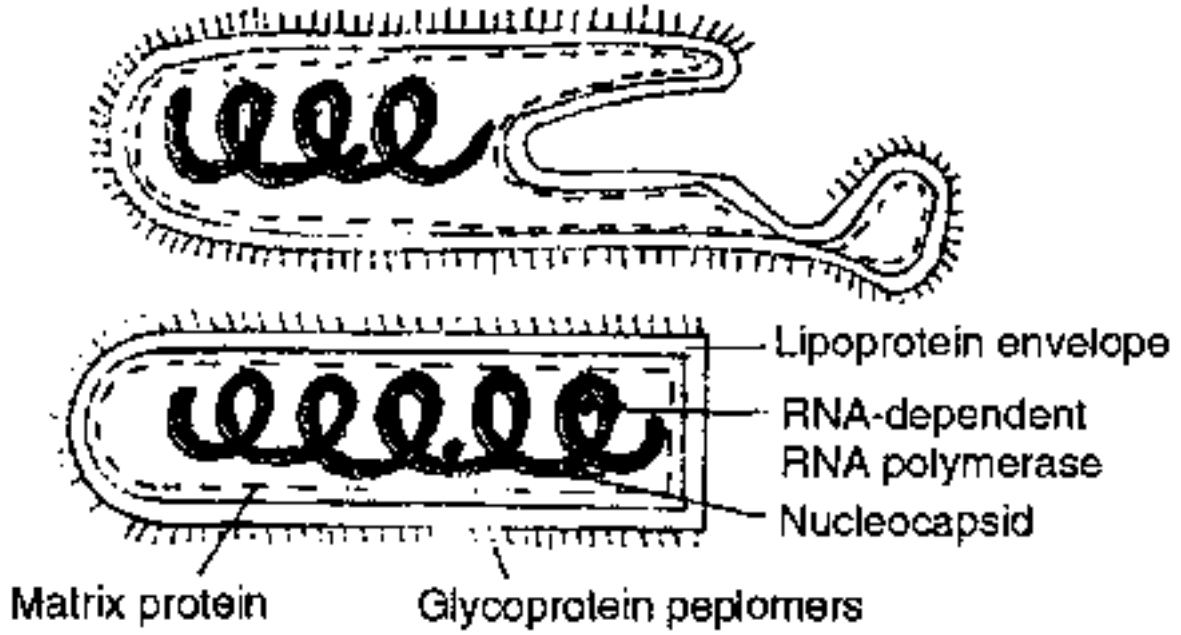


Fig. 6 : Rabies virus

Transmission: The portal of entry from the infected saliva of a rabid animals are:

- abrasions or scratches on the skin.
- mucous membrane exposed to saliva from licks.
- most frequently via deep penetrating bite wounds uncommon routes include:
- inhalation while in bat infested areas/ caves
- aerosols released during centrifugation of infected material in the laboratory.
- ingestion of flesh of rapid animals.
- corneal transplants.
- Humans do not figure as spreaders of rabies.

Epidemiology

Rabies virus is present in animals in all parts of the world except Australia and Antarctica, and some islands like Britain. Two epidemiological types of rabies exist-urban, transmitted by domestic animals like dogs and cats, and sylva tic, involving animals in the wild, such as jackals, wolves, foxes, mongooses, skunks and bats. Most cases of human rabies follow dog bites but in endemic areas almost any animal can transmit rabies. In India, antirabic treatment is to be

considered following the bite of any animal except rats. Where urban or domestic rabies has been controlled, as in the USA, the majority of infections are due to bites by wild animals.

Difference between Prokaryote and Eukaryote

Prokaryotes	Eukaryotes
Circular DNA (in cytosol)	Linear DNA (in nucleus)
No organelles	Several membrane bound organelles
Nucleoid (not membrane bound)	Nucleus (membrane bound)
Single chromosome	Several chromosomes
Plasma membrane typically lacks receptors	Plasma membrane with receptors (sterols and carbohydrates)
Chemically complex cell wall (may contain peptidoglycan)	Chemically simple cell walls (cellulose (plants) and chitin (fungi))
DNA transctription and mRNA translation occurs simultaneously (in cytosol)	DNA transctription in nucleus, and mRNA translation in cytosol
Flagellum (if present) Simple, built from two proteins	Flagellum (if present) Complex, built from microtubules
May have pili and fimbriae	May have cilia
Haploid genome (only one copy of each gene)	Diploid genome (more than one copy of each gene)
May have plasmids (DNA outside chromosome)	Plasmid DNA not common
Compact genome (little repetitive DNA)	Usually large amounts of non-coding and repetitive DNA
May have a glycocalyx cover	Glycocalyx only if no cell wall
Small ribosomes	Large ribosomes in cytosol/nucleus small ribosomes in organelles
No histones in chromosome	DNA "wound" around histones
Lacks cytoskeleton	Cytoskeleton (actin, microtubules)
Mycolaginous capsule	No mycolaginous capsule
Cell size range 0.5–100 µm	Cell size range 10–150 µm
Asexual reproduction (binary fission)	Sexual reproduction (meiosis and mitosis)

UNIT - III

CULTURE MEDIA – PREPARATION AND TYPES

Basic concept of growth media;

Culture media contains nutrients and physical growth parameters necessary for microbial growth. All microorganisms cannot grow in a single culture medium and in fact many can't grow in any known culture medium.

Organisms that cannot grow in artificial culture medium are known as obligate parasites. Mycobacterium leprae, rickettsia, Chlamydia, and Treponema pallidum are obligate parasites. Bacterial culture media can be distinguished on the basis of composition, consistency and purpose.

CLASSIFICATION OF CULTURE MEDIA USED IN MICROBIOLOGY LABORATORY ON THE BASIS OF CONSISTENCY - PHYSICAL

Solid medium

Solid medium contains agar at a concentration of 1.5-2.0% or some other, mostly inert solidifying agent. Solid medium has physical structure and allows bacteria to grow in physically informative or useful ways (e.g. as colonies or in streaks). Solid medium is useful for isolating bacteria or for determining the colony characteristics of the isolate.

Semisolid media

They are prepared with agar at concentrations of 0.5% or less. They have soft custard like consistency and are useful for the cultivation of microaerophilic bacteria or for determination of bacterial motility.

Liquid (Broth) medium

These media contains specific amounts of nutrients but don't have trace of gelling agents such as gelatin or agar. Broth medium serves various purposes such as propagation of large number of organisms, fermentation studies, and various other tests. e.g. sugar fermentation tests, MR-VP broth.

Classification of culture media based on the basis of composition

CHEMICAL

1. Synthetic or chemically defined medium:

A chemically defined medium is one prepared from purified ingredients and therefore whose exact composition is known Synthetic medium.

2. Non synthetic or chemically undefined medium:

Non-synthetic medium contains at least one component that is neither purified nor completely characterized nor even completely consistent from batch to batch. For example, is derived from cultures of yeasts.

Classification of Bacterial Culture Media based on the basis of purpose/ functional use/ application many special purpose media are needed to facilitate recognition, enumeration, and isolation of certain types of bacteria. To meet these needs, numerous media are available.

1. General purpose media/ Basic media:

Basal media are basically simple media that supports most non-fastidious bacteria. Peptone water, nutrient broth and nutrient agar are considered as basal medium. These media are generally used for the primary isolation of microorganisms.

2. Enriched medium (Added growth factors):

- Addition of extra nutrients in the form of blood, serum, egg yolk etc, to basal medium makes them enriched media.
- Enriched media are used to grow nutritionally exacting (fastidious) bacteria.
- Blood agar, chocolate agar, Loeffler's serum slope etc are few of the enriched media.
- Blood agar is prepared by adding 5-10% (by volume) blood to a blood agar base. Chocolate agar is also known as heated blood agar or lysed Blood agar

3. Selective and enrichment media

- They are designed to inhibit unwanted commensal or contaminating bacteria and help to recover pathogen from a mixture of bacteria.
- While selective media are agar based, enrichment media are liquid in consistency. Both these media serve the same purpose.
- Any agar media can be made selective by addition of certain inhibitory agents that don't affect the pathogen of interest.
- Various approaches to make a medium selective include addition of antibiotics, dyes, chemicals, alteration of pH or a combination of these.

a. Selective medium

Principle:

Differential growth suppression selective medium is designed to suppress the growth of some microorganisms while allowing the growth of others. Selective medium is agar based (solid) medium so that individual colonies may be isolated.

Examples of selective media include:

- Thayer martin agar used to recover N.gonorrhoeae contains antibiotics; vancomycin, colistin and nystatin.
- Mannitol salt agar and Salt Milk Agar used to recover S.aureus contains 10% NaCl.
- Potassium tellurite medium used to recover C.diphtheriae contains 0.04% potassium tellurite.
- MacConkey's Agar used for Enterobacteriae members contains bile salt that inhibits most gram positive bacteria.
- Pseudosel Agar (Cetrimide Agar) used to recover P. aeruginosa contains cetrimide (antiseptic agent).
- Crystal Violet Blood Agar used to recover S. pyogenes contains 0.0002% crystal violet.
- Lowenstein Jensen Medium used to recover M.tuberculosis is made selective by incorporating malachite green.
- Wilson and Blair's Agar for recovering S. typhi is rendered selective by the addition of dye brilliant green.
- Selective media such as TCBS Medium used for isolating V. cholerae from fecal specimens have elevated pH (8.5-8.6), which inhibits most other bacteria.

b. Enrichment culture medium

Enrichment medium is used to increase the relative concentration of certain microorganisms in the culture prior to plating on solid selective medium.

- Unlike selective media, enrichment culture is typically used as broth medium. Enrichment media are liquid media that also serves to inhibit commensals in the clinical specimen.
- Selenite F broth, tetrathionate broth and alkaline peptone water are used to recover pathogens from fecal specimens.

4. Differential/ indicator medium: differential appearance:

Certain media are designed in such a way that different bacteria can be recognized on the basis of their colony colour.

- Various approaches include incorporation of dyes, metabolic substrates etc, so that those bacteria that utilize them appear as differently coloured colonies.

- Such media are called differential media or indicator media. Differential media allow the growth of more than one microorganism of interest but with morphologically distinguishable colonies.

Examples of differential media include:

- Mannitol salts agar (mannitol fermentation = yellow)
- Blood agar (various kinds of hemolysis i.e. α, β and γ hemolysis)
- Mac conkey agar(lactose fermenters, pink colonies whereas non- lactose fermenter produces pale or colourless colonies.
- TCBS (Vibrio cholerae produces yellow colonies due to fermentation of sucrose)

5. Transport media:

- Clinical specimens must be transported to the laboratory immediately after collection to prevent overgrowth of contaminating organisms or commensals.
- This can be achieved by using transport media. Such media prevent drying (desiccation) of specimen, maintain the pathogen to commensal ratio and inhibit overgrowth of unwanted bacteria.
- Some of these media (Stuart's & Amie's) are semi-solid in consistency. Addition of charcoal serves to neutralize inhibitory factors.
- Cary Blair transport medium and VenkatramanRamakrishnan (VR) medium are used to transport feaces from suspected cholera patients.

6. Anaerobic media:

Anaerobic bacteria need special media for growth because they need low oxygen content, reduced oxidation –reduction potential and extra nutrients.

- Media for anaerobes may have to be supplemented with nutrients like hemin and vitamin K. Such media may also have to be reduced by physical or chemical means.
- Boiling the medium serves to expel any dissolved oxygen. Addition of 1% glucose, 0.1% thioglycollate, 0.1% ascorbic acid, 0.05% cysteine or red hot iron filings can render a medium reduced.
- Thioglycollate broth contains sodium thioglycollate, glucose, cystine, yeast extract and casein hydrolysate.
- Methylene blue or resazurin is an oxidation-reduction potential indicator that is incorporated in the medium. Under reduced condition, methylene blue is colourless.

7. Assay media:

These media are used for the assay of vitamins, amino acids and antibiotics. E.g. antibiotic assay media are used for determining antibiotic potency by the microbiological assay technique. Other types of medium includes

PURE CULTURE TECHNIQUES

Obtaining a pure culture of bacteria is usually accomplished by spreading bacteria on the surface of a solid medium so that a single cell occupies an isolated portion of the agar surface. This single cell will go through repeated multiplication to produce a visible colony of similar cells, or clones.

The pour plate method is a plating technique that is commonly used for obligate and anaerobic bacteria. This technique is used to isolate microbial colonies by serial dilution and then counting the colony forming units (CFUs). In this method, the liquid sample is poured into the petri dish before the solidification of the agar medium. After solidification, colonies grow both inside and on the surface of the medium. However, the colonies growing inside the medium are confluent; those on the surface are used for viable counting.

Pour Plate Method Principle

The pour plate method is based on the principle of counting viable colonies of microorganisms using serial dilution. A serially diluted sample (usually 1 ml) is poured into the petri dish, and molten agar at 45-50°C is added to the dish and swirled. After solidification, the plate is incubated at an optimal temperature. Viable microbial colonies can be observed on the plate after incubation that can be counted. The CFU/ml can be obtained by formula.

Pour Plate Method Procedure

- Sterilise all the instruments, flasks, and media that are required for the streaking procedure.
- Clean your work area using a disinfectant to minimise any contamination.
- Set up the Bunsen burner in your work area carefully.
- Wash your hands with an antiseptic solution before handling any microbial solution.
- Label the petri dish with all important information, such as your name, date, media used, and the culture being inoculated.
- Sample Preparation: If the sample is in semisolid or solid form, suspend it in sterile water or broth to prepare a liquid solution. If the sample is already in liquid form, prepare serial dilutions of the sample to lessen the load of microbial colonies in the range of 20-300 CFU/ml. You can prepare dilutions up to 10-10.
- For inoculation, open the lids of the Petri dishes and pour 1 ml of the diluted sample. Take the molten agar, heat it a little and pour around 15-18 ml of it onto the sample. Keep in mind

that the agar should not be either too hot or too cold. Close the lid of the dish and swirl it slowly.

- Another method for inoculation is to mix the diluted sample in the agar medium, mix it gently, and then pour it into the petri dish. However, this method is less commonly used.
- Let the plate solidify.
- Invert the plate and incubate it at an optimal temperature (usually 37℃) for 24-48 hours.

Interpretation

After incubation, the plates are observed for viable colony counts. If all the colonies look alike, it indicates that only one bacterial species is present on the plate. However, if different colonies are seen, it is either different species that have grown on the plate or there is some kind of contamination.

For obtaining the CFU/ml, count the colonies on the plate, and use the above formula to get the value. The optimum count of colonies obtained must be between 20-300 CFU/ml. If it is more or less than this range, the whole process needs to be repeated.

Applications of the Pour Plate Method

- It is used by scientists to obtain microbial growth curves and in the calculation of the concentration of cells in a particular sample.
- It is also used to check the effect of various growth factors and environmental factors on the growth rate of the bacteria.

Advantages of the Pour Plate Method

- It is useful for counting viable colonies.
- It can detect very low loads of bacterial counts as well.
- It does not require previously solidified agar plates.
- It can also be used for clinical and environmental samples.

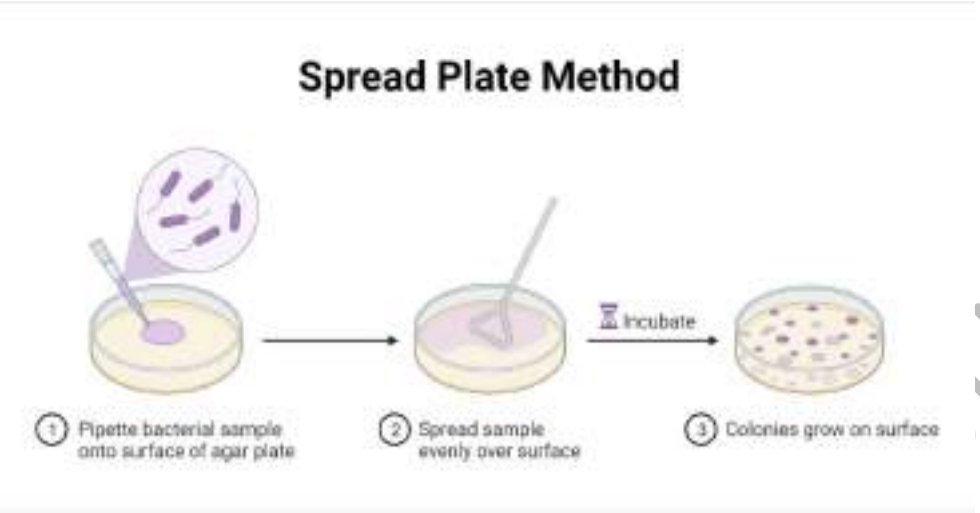
SPREAD PLATE

The spread plate method is a microbiological laboratory technique for isolating and counting the viable microorganisms present in a liquid sample by spreading a certain volume of the sample over an appropriate solidified culture media.

Following the incubation, in a successful spread plate, there will be the formation of evenly distributed discrete colonies all over the surface of the culture media.

This technique is used for isolating and counting the total number of viable microorganisms (i.e. calculating the colony-forming units per mL (CFU/mL) in the given sample. It is also for

propagating the old culture and mass producing them. It can be used for all of the culturable bacteria and fungi.



Spread Plate Method

The sample in the spread plate method must be liquid or in suspension. Before plating, the samples are serially diluted. If the objective is to count the CFU/mL then the sample must be diluted to make the microbial load in the sample between 20 – 300 CFU/mL (suitable colony counting range is 20 – 200, some consider it to be 30 – 300, and in average it is taken as 25 – 250). This can be obtained by pilot test or by using a different range of diluted samples. If the sample is solid or semisolid, it must be first emulsified and then serially diluted to reduce microbial load up to the permitted range.

0.1 mL of the sample (0.1 to 0.2 mL) is pipetted over the center of the solidified agar medium and evenly spread over the surface of the medium. The plates are incubated under the optimum condition following which the numbers of colonies are counted. If the colonies are uncountable or fused or more than 300 CFU/mL or less than 20 CFU/mL, it is recommended to repeat the process for getting the optimum count.

STREAK PLATE

Streaking is a technique used in microbiology for the isolation of single colonies of microorganisms, either from a mixed species or from the same species. This technique is mostly applicable to bacteria but is also used for some yeasts. It is an old technique that has been in use since the time of Robert Koch. It was first demonstrated by Loeffler and Gaffky in Koch's laboratory.

Streak Plate Method Principle

The streak plate method is based on the principle of dilution. It can be described as a rapid qualitative isolation technique. The main criterion of isolation is to obtain a reduced number of colonies. In this technique, a loopful of culture is spread on an agar plate to get individual cells far apart enough from each other. The streaking method gradually dilutes the inoculum such that the bacterial cells can be counted as colony forming units (CFUs).

Types of Streak Plate Method

1. **Quadrant Streaking:** This is the most common method of streaking, where the petri dish is divided into four quadrants and then inoculated. It is also known as a four-quadrant streak. A loopful of inoculum is taken and streaked such that the first quadrant contains the highest concentration of the inoculum, followed by the second quadrant, third quadrant, and fourth quadrant.

This is a discontinuous method where the loop is sterilised after streaking in every quadrant. By the time the fourth quadrant is streaked, the inoculum is diluted enough to give rise to individual colonies.

1. **T-Streaking:** In this method, the petri dish is divided into three quadrants by drawing the letter 'T'. Similar to four-quadrant streaking, it is a discontinuous method where each quadrant is streaked after loop sterilisation, and the quadrant that is streaked last gives isolated colonies.

2. **Continuous Streaking**: In this type of streaking, the inoculum is spread from one edge to the centre of the plate. The plate is rotated 180°, and the remaining portion is streaked without sterilising the loop.

Another form of continuous streaking is used for diagnostic purposes. The petri dish is divided into sections, and different cultures, such as urine, sputum, pus, etc., are streaked in each section to get maximum output.

1. **Radiant Streaking:** In this streaking method, the inoculum is spread on one edge of the petri dish. From the edge, vertical lines are streaked in the upward direction. Next, the vertical lines are streaked horizontally to obtain pure isolated cultures.

Streak Plate Method Procedure

- Sterilise all the instruments, flasks and media that are required for the streaking procedure.
- Clean your work area using a disinfectant to minimise any contamination.
- Set up the bunsen burner in your work area carefully.
- Wash your hands with an antiseptic solution before handling any microbial solution.

- Label the petri dish with all important information, such as your name, date, media used and the culture being inoculated.
- To pick up the sample, you can use either a metal loop or disposable plastic loops.
- A loopful of sample is streaked on the first quadrant in a back-and-forth motion on the agar plate.
- Sterilise the loop by heating it in the bunsen burner if using a metal loop.
- Streak the other three quadrants by a similar method.
- Close the lid of the plate after streaking, and store the dish upside down in an incubator with optimal temperature.

Advantages of the Streak Plate Method

- It is one of the most popular techniques used to obtain isolated colonies of bacteria.
- It finds a great application in biotechnology as it can be used to identify transformed bacteria from non-transformed bacteria by adding an antibiotic to the growth medium.
- It also finds great application in diagnostic purposes.

BACTERIAL GROWTH

Bacterial growth is a complex process that involves numerous anabolic and catabolic reactions, which result in cell division. The increase in numbers or bacterial mass can be measured as a function of time under pure culture conditions, where the nutrients and environmental conditions are controlled. Growth of bacterial cultures is defined as an increase in the number of bacteria in a population rather than in the size of individual cells. The growth of a bacterial population occurs in a geometric or exponential manner: with each division cycle (generation), one cell gives rise to 2 cells, then 4 cells, then 8 cells, then 16, then 32, and so forth. Typically bacteria divides by Binary fission by a series of steps

Fission in Bacteria.

1. Prokaryote cells grow by increasing in cell number (as opposed to increasing in size).
2. Replication is by binary fission, the splitting of one cell into two.
3. Therefore, bacterial populations increase by a factor of two (double) every generation time.

Generation time : The time required to for a population to double (doubling time) in number.

Ex. Escherichia coli (E. coli) double every 20 minutes

Ex. Mycobacterium tuberculosis double every 12 to 24 hours

Growth in Batch Culture:

The growth of bacteria (or other microorganisms, as protozoa, microalgae or yeasts) in batch culture can be studied with four different phases: lag phase (A), log phase or exponential phase (B), stationary phase (C), and death phase (D).

1. During lag phase, bacteria adapt themselves to growth conditions. It is the period where the individual bacteria are maturing and not yet able to divide. During the lag phase of the bacterial growth cycle, synthesis of RNA, enzymes and other molecules occurs. During the lag phase cells change very little because the cells do not immediately reproduce in a new medium. This period of little to no cell division is called the lag phase and can last for 1 hour to several days. During this phase cells are not dormant.

2. The log phase (sometimes called the logarithmic phase or the exponential phase) is a period characterized by cell doubling. The number of new bacteria appearing per unit time is proportional to the present population. If growth is not limited, doubling will continue at a constant rate so both the number of cells and the rate of population increase doubles with each consecutive time period. For this type of exponential growth, plotting the natural logarithm of cell number against time produces a straight line. The slope of this line is the specific growth rate of the organism, which is a measure of the number of divisions per cell per unit time. The actual rate of this growth (i.e. the slope of the line in the figure) depends upon the growth conditions, which affect the frequency of cell division events and the probability of both daughter cells surviving. Under controlled conditions, cyanobacteria can double their population four times a day and then they can triple their population. Exponential growth cannot continue indefinitely, however, because the medium is soon depleted of nutrients and enriched with wastes.

3. The stationary phase is often due to a growth-limiting factor such as the depletion of an essential nutrient, and/or the formation of an inhibitory product such as an organic acid. Stationary phase results from a situation in which growth rate and death rate are equal. The number of new cells created is limited by the growth factor and as a result the rate of cell growth matches the rate of cell death. The result is a “smooth,” horizontal linear part of the curve during the stationary phase. Mutations can occur during stationary phase. Bridges et al. (2001) presented evidence that DNA damage is responsible for many of the mutations arising in the genomes of stationary phase or starving bacteria. Endogenously generated reactive oxygen species appear to be a major source of such damages.

4. Death phase (Decline phase) bacteria die. This could be caused by lack of nutrients, environmental temperature above or below the tolerance band for the species, or other injurious conditions. This basic batch culture growth model draws out and emphasizes aspects of bacterial growth which may differ from the growth of macrofauna. It emphasizes clonality,

asexual binary division, the short development time relative to replication itself, the seemingly low death rate, the need to move from a dormant state to a reproductive state or to condition the media, and finally, the tendency of lab adapted strains to exhaust their nutrients. In reality, even in batch culture, the four phases are not well defined. The cells do not reproduce in synchrony without explicit and continual prompting (as in experiments with stalked bacteria and their exponential phase growth is often not ever a constant rate, but instead a slowly decaying rate, a constant stochastic response to pressures both to reproduce and to go dormant in the face of declining nutrient concentrations and increasing waste concentrations.

Batch culture is the most common laboratory growth method in which bacterial growth is studied, but it is only one of many. It is ideally spatially unstructured and temporally structured. The bacterial culture is incubated in a closed vessel with a single batch of medium. In some experimental regimes, some of the bacterial culture is periodically removed and added to fresh sterile medium. In the extreme case, this leads to the continual renewal of the nutrients. This is a chemostat, also known as continuous culture. It is ideally spatially unstructured and temporally unstructured, in a steady state defined by the rates of nutrient supply and bacterial growth. In comparison to batch culture, bacteria are maintained in exponential growth phase, and the growth rate of the bacteria is known. Related devices include turbidostats and auxostats. When Escherichia coli is growing very slowly with a doubling time of 16 hours in a chemostat most cells have a single chromosome.

Bacterial growth can be suppressed with bacteriostats, without necessarily killing the bacteria. In a synecological, true-to-nature situation in which more than one bacterial species is present, the growth of microbes is more dynamic and continual.

Liquid is not the only laboratory environment for bacterial growth. Spatially structured environments such as biofilms or agar surfaces present additional complex growth models.

Growth of bacteria in a batch culture can be summarized as below:

1. Bacteria growing in batch culture produce a growth curve with up to four distinct phases.
2. Batch cultures are grown in tubes or flasks and are closed systems where no fresh nutrients are added or waste products removed.
3. Lag phase occurs when bacteria are adjusting to them medium. For example, with a nutritionally poor medium, several anabolic pathways need to be turned on, resulting in a lag before active growth begins.
4. In log or exponential phase, the cells are growing as fast as they can, limited only by growth conditions and genetic potential. During this phase, almost all cells are alive, they are most nearly identical, and they are most affected by outside influences like disinfectants.
5. Due to nutrient depletion and/or accumulation of toxic end products, replication stops and cells enter a stationary phase where there is no net change in cell number.

6. Death phase occurs when cells can no longer maintain viability and numbers decrease as a proportion

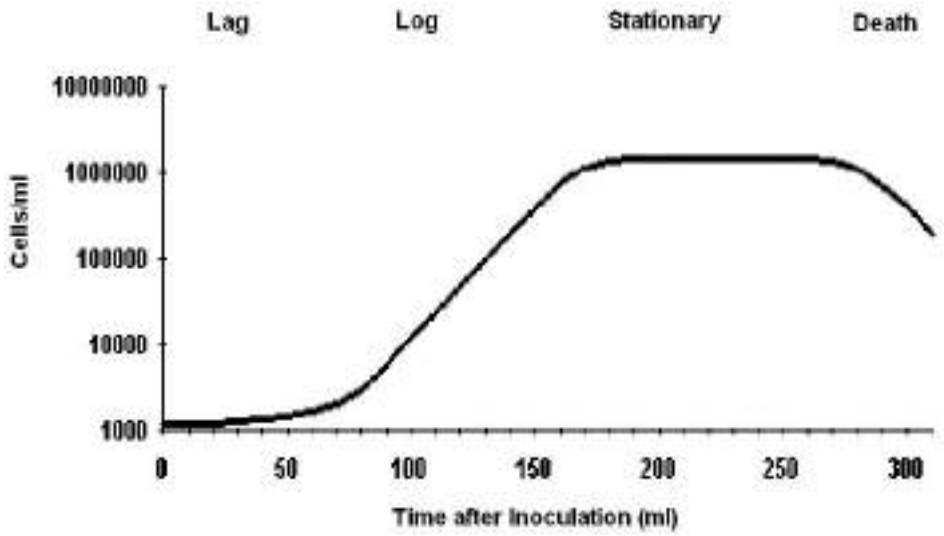


Fig :Growth in Batch Culture

Mean Generation Time and Growth Rate:

The mean generation time (doubling time) is the amount of time required for the concentration of cells to double during the log stage. It is expressed in units of minutes.

Growth rate (min) = -1

Mean generation time can be determined directly from a semi log plot of bacteria lconcentration vs time after inoculation

Environmental factors affecting growth: Various factors affects growth below:

- Temperature
- Oxygen requirement
- pH
- Water availability

Temperature:

Enzymes, the machinery of the cell, are influenced by external factors and can be shown to have a range where they function that includes an optimal value that produces the highest activity.The range of enzyme activity determines the range for growth of specific bacteria, analogously leading to a value for optimal growth rate. In the case of temperature, bacteria are divided into categories based on the temperature range where they can grow and the temperature that provides optimal growth:

A. **Psychrophile/cryophiles:** Psychrophiles or cryophiles (adj. psychrophilic or cryophilic) are extremophilic organisms that are capable of growth and reproduction in low temperatures, ranging from −20 °C to +10 °C. They are found in places that are permanently cold, such as the polar regions and the deep sea. Psychrophile is Greek words means 'cold loving'. Many such organisms are bacteria or archaea, but some eukaryotes such as lichens, snow algae, fungi, and wingless midges, are also classified as psychrophiles.

B. **Psychrotroph:** Psychrotrophs are cold-tolerant bacteria that have the ability to grow at low temperatures but have optimal and maximal growth temperatures above 15°C and 20°C, respectively. This type of organisms can survive below temperature less than 20°C to 30°C. Important in food spoilage.

C. **Mesophile:** A mesophile is an organism that grows best in moderate temperature, neither too hot nor too cold, with an optimum growth range from 20 to 45 °C (68 to 113 °F). They are More common and are disease causing

D. **Thermophiles:** A thermophile is an organism—a type of extremophile—that thrives at relatively high temperatures, between 41 and 122 °C (106 and 252 °F). Many thermophiles are archaea. Thermophilic eubacteria are suggested to have been among the earliest bacteria. Thermophiles are found in various geothermally heated regions of the Earth, such as hot springs like and deep sea hydrothermal vents, as well as decaying plant matter, such as peat bogs and compost. Thermophiles can survive at high temperatures, whereas other bacteria would be damaged and sometimes killed if exposed to the same temperatures. The enzymes in thermophiles function at high temperatures. Some of these enzymes are used in molecular biology, for example the taq polymerase used in PCR. "Thermophile" is derived from the Greek: θερμότητα (thermotita), meaning heat, and Greek: φίλια (philia), love.

E. **Hyperthermophiles**: A hyperthermophile is an organism that thrives in extremely hot environments—from 60 °C (140 °F) upwards. An optimal temperature for the existence of hyperthermophiles is often above 80 °C (176 °F). Hyperthermophiles are often within the domain Archaea, although some bacteria are able to tolerate temperatures of around 100 °C (212 °F), as well. Some bacteria can live at temperatures higher than 100 °C at large depths in sea where water does not boil because of high pressure. Many hyperthermophiles

are also able to withstand other environmental extremes such as high acidity or high radiation levels. Hyperthermophiles are a subset of extremophiles. It is believed that the cell structure of these type of bacteria contain high level of saturated fatty acid that retain its shape at high temperature.

ANAEROBIC CULTURE TECHNIQUES

Microbial culture is used to multiply and study microorganisms by providing them with a controlled nutrient media and under ideal laboratory conditions. The major constituents of a culture media can be listed as follows:

- Energy source
- Carbon source
- Salts
- Nitrogen source
- pH
- Growth factors etc.

The aerobic and anaerobic culture methods are distinguished based on growth in the presence of oxygen. They are:

- Aerobes- These are organisms that can grow up to some extent in the presence of oxygen.
- Obligate Aerobes- Organisms that cannot grow in the absence of oxygen.
- Anaerobes- These are organisms that generally grow well in low oxygen content environments.
- Obligate anaerobes- Organisms that die in the presence of oxygen and grow in extreme oxygen-less environments.

Aerobic Culture Methods:

The growth media is selected based on the type of microbe to be cultured. A rich and complete media is generally used for growing a pure (containing a particular strain of the microbe) microbial line in bulk. At the same time, minimal media is essential for regulating a specific pathway in the microbe. We should also consider that these cultures need to be grown in sterile conditions. The growth phase of bacteria can be divided into four stages:

- Lag Phase-The bacteria adjust themselves to their new surroundings.
- Log Phase- This is the growth phase of the culture. The bacteria divide rapidly to multiply at an exponential rate.
- Stationary Phase-Due to nutrient utilization and waste material build-up, the growth rate slows down to become equal to the death phase.

- Death Phase- Due to unfavourable conditions, there is a steady decline in the number of cells, and the amount of death surpasses the amount of bacterial growth.

Anaerobic Culture Methods:

Anaerobic bacteria have to be cultivated in the absence of oxygen. Thus proper care has to be taken to ensure it. The culture media generally consists of brain-heart infusion, vitamin K, and amino acids. The use of culture media depends on the type of specimen of bacteria. The cultures are grown in an oxygen-free environment at 95 degrees F for 48 hours. Entirely sealed cabinets of plastic are used to produce the microbes. The interior composition of gases generally consists of 10% hydrogen, 10% carbon dioxide and 80% nitrogen.

Applications of Microbial Culture:

Once the microbe is grown using aerobic and anaerobic culture methods reaches a phase of stable growth, they can be used for a variety of purposes such as:

- **Diagnosis:** Culturing microbes and testing them subsequently to identify the microbe can help us identify the cause of a disease. This is of prime medical importance. The samples for such study can be extracted from wounds, blood, urine and other body fluids.
- **Drug Discovery:** The vast array of antibiotics available in the market would not have been possible without the culturing and further testing of bacteria in Petri dishes. The vast library of possible antibiotic targets is tested after culturing bacteria to select potential drug candidates. This is a long and cost-intensive process.
- **Studying microbial colonies:** It has been observed that microbes can behave differently in colonies instead of when they are present individually. Such studies have proven that there is a constant cross-talk between neighbouring microbes. They communicate with the help of quorum sensing, sporulation, contact signalling etc.
- **Creating models:** Recombinant DNA Technology has allowed us to insert certain genes and study their interactions in microbes. This allows us to understand the functional aspect of the gene using knockout studies. So the creation of such models has helped us to form a holistic understanding of the genome.

Anaerobic Bacteria Test Methods:

Anaerobic and aerobic microbes can be separated by first culturing them using aerobic and anaerobic culture methods in a test tube of thioglycollate broth. Now, based on the position of the growth of the microbe, we can classify them:

- Obligate aerobes grow at the top.
- Obligate anaerobes gather at the bottom of the tube.

- Facultative anaerobes are present everywhere but are present at a higher concentration at the top
- Microaerophiles gather at the upper part of the test tube but not at the top
- Aerotolerant organisms are distributed evenly throughout the test tube.

Thus, by following this test, we can easily distinguish our anaerobic microbe

UNIT – IV

MICROORGANISMS

Discovery of Microorganisms:

The first microorganism was observed by Antony van Leeuwenhoek (1632-1723) by his simple made microscope which was nothing but assembly of glasses. He called them as "Animalcules".

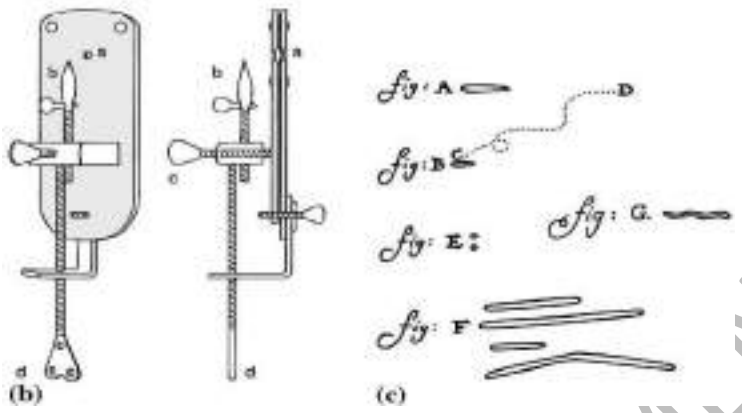


Fig. First Microscope prepraed by A.V.Leewenhook and structure of Microorganism.

Lenses and the Bending of Light: The property of a Microscope depends on the light property, Lenses and bending of light. The common properties of lights are:

- Light is refracted (bent) when passing from one medium to another.
- The bending of light is the property of Refractive indexes of different media.
- A measure of how greatly a substance slows the velocity of light.
- Direction and Magnitude of bending is determined by the refractive indexes of the two media forming the interface.

Lenses:

- Focus light rays at a specific place called the focal point.
- Distance between centre of lens and focal point is the focal length.
- Strength of lens related to focal length.
- Short focal length more magnification.

The Light Microscope: The light microscope is an important tool in the study of microorganisms, particularly for identification purposes.

Principles

The light microscope is an instrument for visualizing fine detail of an object. It does this by creating a magnified image through the use of a series of glass lenses, which first focus a beam of light onto or through an object, and convex objective lenses to enlarge the image formed. In the majority of light microscopes, the image is viewed directly through binocular eyepieces that act as a secondary lens in the form of a magnifying glass to observe the projected image. Such instruments are termed 'compound microscopes,' and the total magnification is the sum of the objective magnification and the eyepiece magnification. The magnification range extends from ×10 to ×1000, with a resolving power of the order of 0.2 µm, depending on the type and numerical aperture (area available for passage of light) of the objective lenses. A number of books are available, providing comprehensive details on the theory of the light microscope and guidance to the practical use of the instrument, including methods of image enhancement and instrument care.

Types of compound Microscope: Based on source of energy used light can be

- Bright-field Microscope.
- Dark-field Microscope.
- Phase-contrast Microscope.
- Fluorescence Microscopes

These are compound microscopes: The compound light microscope uses visible light to directly illuminate specimens in a two-lens system, resulting in the illuminated specimen appearing dark against a bright background. The two lenses present in a compound microscope are the ocular lens in the eyepiece and the objective lens located in the revolving nosepiece. The image is formed by the action of 2 lenses

The Bright-Field Microscope: Produces a dark image against a brighter background. It has several objective lenses. Parfocal microscopes remain in focus when objectives are change. Total magnification is the product of the magnifications of the ocular lens and the objective lens

Fig : A compound Microscope.

Parts of Compound Microscope: A typical Compound Microscope have following parts

1. Illuminator: the light source in the base of the microscope;
2. Abbe Condensor: a two lens system that collects and concentrates light from the illuminator and directs it to the iris diaphragm;
3. Iris diaphragm: regulates the amount of light entering the lens system;
4. Mechanical stage: a platform used to place the slide on which has a hole in the center to let light from the illuminator pass through. Often contains stage clips to hold the slide in place;
5. Body tube: houses the lens system that magnifies the specimens;
6. Upper end of body tube—oculars/eye pieces: what you view through;
7. Lower end of body tube—nose-piece: revolves and contains the objectives.

Essentially, a light microscope magnifies small objects and makes them visible. The science of microscopy is based on the following concepts and principles:

a) Magnification is simply the enlargement of the specimen. In a compound lens system, each lens sequentially enlarges or magnifies the specimen;

b) The objective lens magnifies the specimen, producing a real image that is then magnified by the ocular lens resulting in the final image;

c) The total magnification can be calculated by multiplying the objective lens value by the ocular lens value.

Microscope Resolution- Ability of a lens to separate or distinguish small objects that are close together. In other word it is defined as the inverse of the distance or angular separation between two objects which can be just resolved when viewed through the optical instrument.

Resolving Power of Microscope:

For microscopes, the resolving power is the inverse of the distance between two objects that can be just resolved. This is given by the famous Abbe's criterion given by Ernst Abbe in 1873 as

$$\Delta d = \lambda 2 n \sin \theta$$

$$\text{Resolving power} = 1\Delta d = 2n \sin \theta\lambda$$

Where n is the refractive index of the medium separating object and aperture. Note that to achieve high-resolution n sin θ must be large. This is known as the Numerical aperture.

Thus, for good resolution:

1. sin θ must be large. To achieve this, the objective lens is kept as close to the specimen as possible.
2. A higher refractive index (n) medium must be used. Oil immersion microscopes use oil to increase the refractive index. Typically for use in biology studies, this is limited to 1.6 to match the refractive index of glass slides used. (This limits reflection from slides). Thus the numerical aperture is limited to just 1.4-1.6. Thus, optical microscopes (if you do the math) can only image to about 0.1 microns. This means that usually organelles, viruses and proteins cannot be imaged.
3. Decreasing the wavelength by using X-rays and gamma rays. While these techniques are used to study inorganic crystals, biological samples are usually damaged by x-rays and hence are not used.

The limit set by Abbe's criterion for optical microscopy cannot be avoided. However, using different fluorescence microscopy techniques the Abbe's limit can be circumvented. Stefan Hell used a technique called Stimulated Emission Depletion (STED) and the duo Eric Betzig and W.E. Moerner used superimposed images using green fluorescent proteins to bypass the resolution limit and obtain optical images in never before seen resolution. All three were awarded the 2014 Nobel Prize in Chemistry for their pioneering work. Wavelength of light used is major factor in resolution shorter wavelength greater resolution

Working distance: It is the distance between the front surface of lens and surface of cover glass or specimen

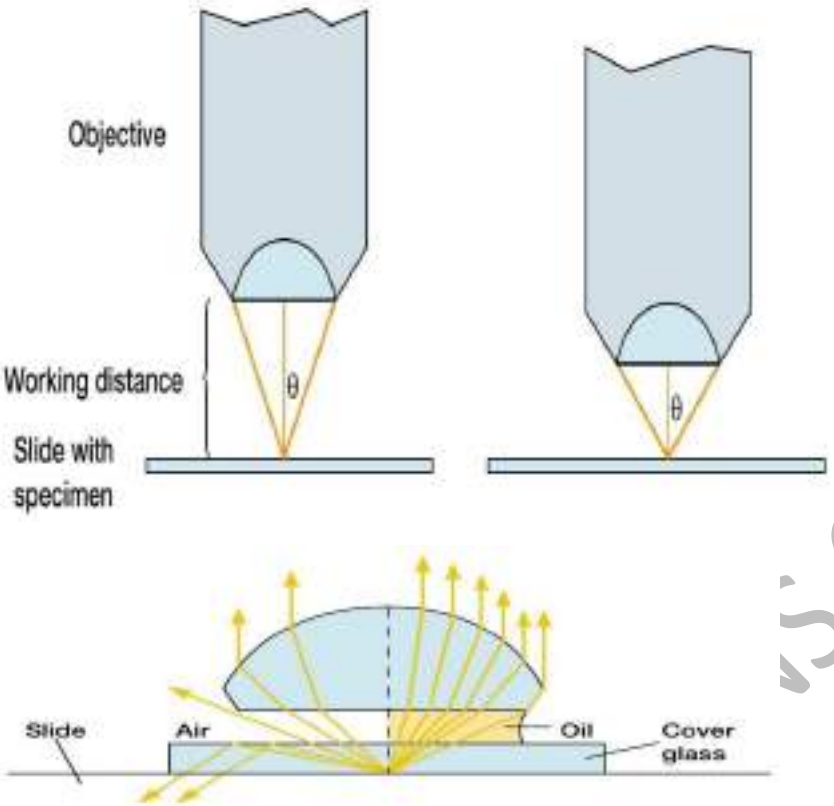


Fig: Working distance and use of immersion oil for better resolution

The Dark-Field Microscope: Produces a bright image of the object against a dark background. Used to observe living, unstained preparations.

Preparation and Staining of Specimens: The main function of stain in microscopy is to Increases visibility of specimen and accentuates specific morphological features of Microorganism. It also preserves the specimens for long time to study later on.

Fixation: It is the process by which internal and external structures are preserved and fixed in position. During fixation the organism under observation is killed and firmly attached to microscope slide. There are two methods for fixation of organisms:

A. **Heat fixing:** Preserves overall morphology but not internal structures
B. **Chemical fixing:** Protects fine cellular substructure and morphology of larger, more delicate organisms

Dyes and Simple Staining

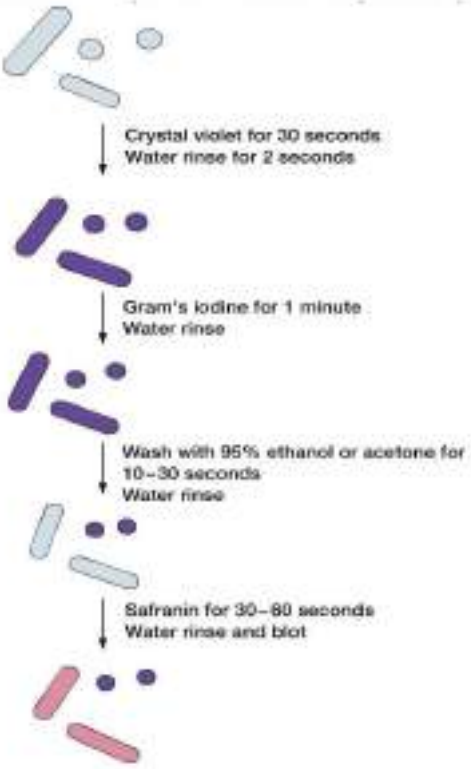
Dyes: Make internal and external structures of cell more visible by increasing contrast with background. It have have two common features. A chromophore groups B. chemical groups with conjugated double bonds give dye its colour and ability to bind cells.

Types of Staining: There are many types of Staining depending upon our objectives of observation:

1. **Simple staining:** In this process a single staining agent is used. Basic dyes are frequently used i.e dyes with positive charges which have ability to bind with negatively charged bacterial surface e.g., crystal violet
2. **Differential Staining:** Divides microorganisms into groups based on their staining properties. e.g., Gram stain (Divide bacteria into gram +ve and Gram –ve) e.g., Acid-fast stain (For Mycobacterium tuberculosis)

Gram staining: most widely used differential staining procedure. Divides Bacteria into two group as Gram +ve and Gram –ve. In this method a series of Chemicals are used to stain the bacteria and then differentiated based on the property to retain a particular dye colour such as crystal violet in gram +ve and Safranin in Gram negative(A counterstain).

The Steps of gram staining is given below:



Flow chart: Process of Gram staining

Source: Wikipedia.org.in

Acid-fast staining: Particularly useful for staining members of the genus Mycobacterium. Mycobacterium tuberculosis – causes tuberculosis, e.g., Mycobacterium leprae – causes leprosy. Mycobacterium have high lipid content in cell walls that is mainly responsible for their staining characteristics.

3. Staining Specific Structures: Some of the dye have ability to stain special structure such as flagella,Endospore,Capsule etc. Some of them are listed below:

4. Negative staining: This staining methods is often used to visualize capsules surrounding bacteria. The capsules looks colourless against a stained background.

Spore staining: For Visualizing Bacterial Endospore Double staining technique is used in which bacterial endospore retain one colour and vegetative cell retain a different colour.

Flagella staining: Flagella are too thin to be visualized using a bright field microscope with ordinary stains, such as the Gram stain, or a simple stain. A wet mount technique is used for staining bacterial flagella, and it is simple and useful when the number and arrangement of flagella are critical to the

identification of species of motile bacteria. The staining procedures require the use of a mordant so that the stain adheres in layers to the flagella, allowing visualization.

Other Microscope:

Phase-contrast microscopy: It is an optical microscope that converts phase shifts in light passing through a transparent specimen to brightness changes in the image. Phase shifts themselves are invisible, but become visible when shown as brightness variations. Its an example of Dark field Microscope. The Microscope was invented by Zernick in 1950.

When light waves travel through a medium other than vacuum, interaction with the medium causes the wave amplitude and phase to change in a manner dependent on properties of the medium. Changes in amplitude (brightness) arise from the scattering and absorption of light, which is often wavelength-dependent and may give rise to colours. Phase-contrast microscopy is particularly important in biology. It reveals many cellular structures that are not visible with a simpler bright-field microscope.

These structures were made visible to earlier microscopes by staining, but this required additional preparation and thus killing the cells. The phase-contrast microscope made it possible for biologists to study living cells and how they proliferate through cell division. It is one of the few methods available to quantify cellular structure and components that does not use fluorescence. After its invention in the early 1930s, phase-contrast microscopy proved to be such an advancement in microscopy that its inventor Frits Zernike was awarded the Nobel Prize in Physics in 1953.

The basic principle to making phase changes visible in phase-contrast microscopy is to separate the illuminating (background) light from the specimen-scattered light (which makes up the foreground details) and to manipulate these differently.

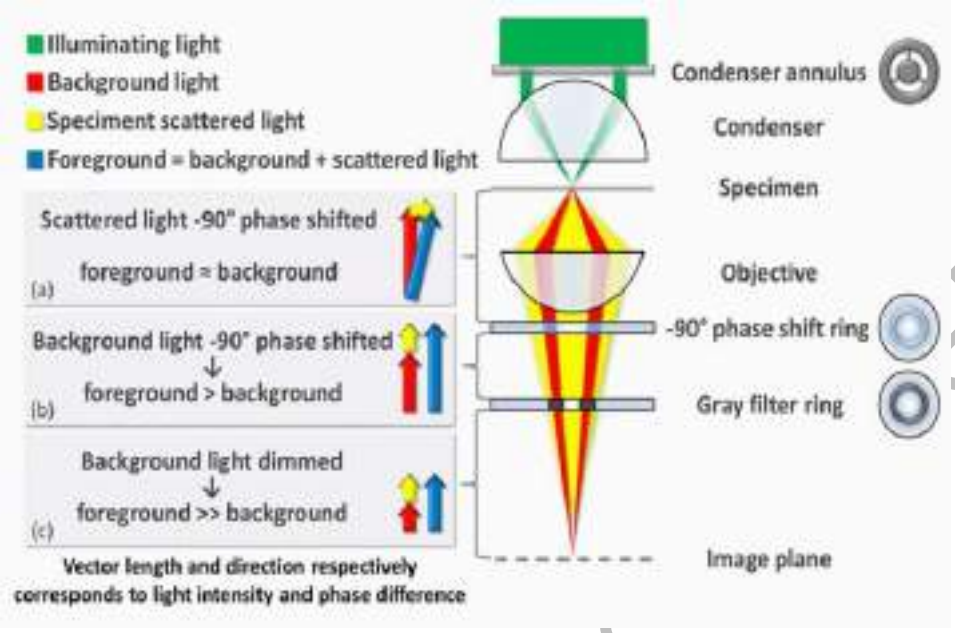


Fig: A Phase Contrast Microscope with working Principal.
Source: Wikipedia.org.in

Fig: Phase Contrast Microscope Olympus Made.

Steps to observe image under Phase Contrast Microscope: Difference in working of A Dark field Microscope and Phase Contrast Microscope:

1. Dark Field Microscope consists of a detector, lenses to magnify, A Phase ring and a light source.
2. The phase ring stops part of the light so that it cannot reach the detector anymore
3. When a sample is inserted, it scatters part of the light even if it is transparent.
4. The scattered light refocused on the detector,

5. The image of the sample appeared on the screen.
6. Dark field allows increasing the contrast of image of unstained and uncoloured objects.

Phase Contrast Microscope function:

1. When the sample is inserted it scatters part of the light which is then also refocused to the detector
2. To distinguish direct light from that of scattered by the sample, a phase plate is inserted.
3. The scattered part crosses a thicker part of the plate. This shift its phase compared to the direct light.
4. Scattered light then interferes with direct light which create a phase contrast.
5. The resulting intensity differences allow to visualize transparent samples

Fluorescence Microscope:

The specimen is illuminated with light of a specific wavelength (or wavelengths) which is absorbed by the fluorophores, causing them to emit light of longer wavelengths (i.e., of a different colour than the absorbed light). The illumination light is separated from the much weaker emitted fluorescence through the use of a spectral emission filter. Typical components of a fluorescence microscope are a light source (xenon arc lamp or mercury-vapor lamp are common; more advanced forms are high-power LEDs and lasers), the excitation filter, the dichroic mirror and the emission filter (see figure below). The filters and the dichroic beam splitter are chosen to match the spectral excitation and emission characteristics of the fluorophore used to label the specimen. In this manner, the distribution of a single fluorophore (colour) is imaged at a time. Multi-colour images of several types of fluorophores must be composed by combining several single-color images.

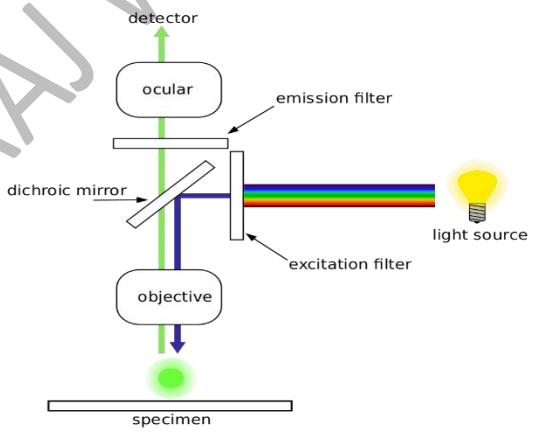


Fig: Working of Flurescence Microscope

Source:Wikipedia.org.in

Fig: An upright fluorescence microscope (Olympus BX61) with the fluorescence filter cube the objective lenses, Coupled with a digital camera.

1. Light is allowed to pass through excitation filter to fall upon Object under investigation (Blue Light).
2. Object reflected the light of higher Wave length (Green light)
3. Detected as green object in two dimensional and three dimensional figure.
4. Even small quantity of cell component can be visualized by this microscope by moving above the ocular lenses over each part of the cell component.

5. **Electron Microscopy**: A beams of electrons are used to produce images. Since wavelength of electron beam is much shorter than light, resulting in much higher resolution. It is that type of microscope that uses a beam of accelerated electrons as a source of illumination. As the wavelength of an electron can be up to 100,000 times shorter than that of visible light photons, electron microscopes have a higher resolving power than light microscopes and can reveal the structure of smaller objects. A scanning transmission electron microscope has achieved better than 50 pm resolution in annular dark-field imaging mode and magnifications of up to about 10,000,000× whereas most light microscopes are limited by diffraction to about 200 nm resolution and useful magnifications below 2000×. Electron microscopes use shaped magnetic fields to form electron optical lens systems that are analogous to the glass lenses of an optical light microscope. It is used to investigate the ultrastructure of a wide range of biological and inorganic specimens, cells, large molecules, metals, and crystals. The First Electron Microscope was developed in 1931 by the physicist Ernst Ruska and the electrical engineer Max Knoll.

Fig: A Typical Electron Microscope. Image of a fly under EM

Electron Microscope are of two types:

Transmission Electron Microscope and Scanning Electron Microscope

A. Transmission electron microscope (TEM), is a known electron Microscope that uses a high voltage electron beam to illuminate the specimen and create an image. The electron beam is produced by an electron gun, commonly fitted with a tungsten filament cathode as the electron source. The electron beam is accelerated by an anode typically at +100 keV (40 to 400 keV) with respect to the cathode, focused by electrostatic and electromagnetic lenses, and transmitted through the specimen that is in part transparent to electrons and in part scatters them out of the beam. When it emerges from the specimen, the electron beam carries information about the structure of the specimen that is magnified by the objective lens system of the microscope. The spatial variation in this information (the "image") may be viewed by projecting the magnified electron image onto a fluorescent viewing screen coated with a phosphor or scintillator material such as zinc sulfide.

Alternatively, the image can be photographically recorded by exposing a photographic film or plate directly to the electron beam, or a high-resolution phosphor may be coupled by means of a lens optical system or a fibre optic light-guide to the sensor of a digital camera. The image detected by the digital camera may be displayed on a monitor or computer.

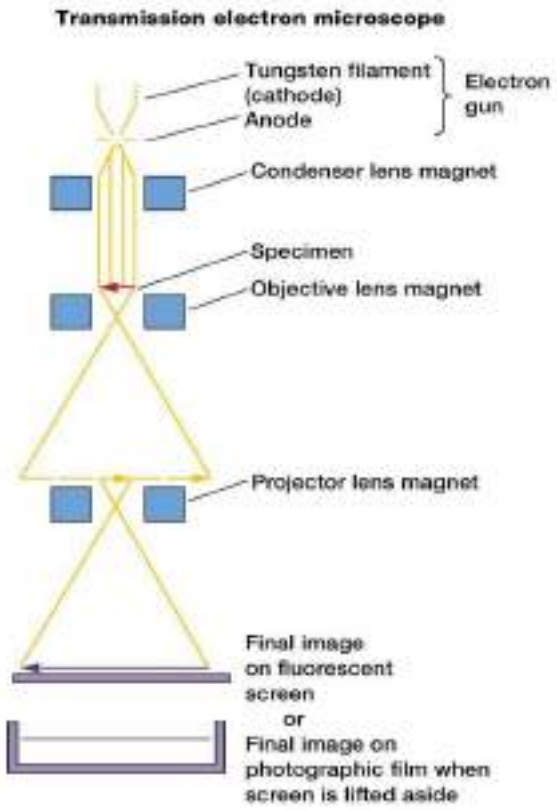


Fig: Working of Transmission Electron Microscope

Specimen Preparation: Analogous to procedures used for light microscopy. For transmission electron microscopy, specimens must be cut very thin. Specimens are chemically fixed and stained with electron dense material

The Scanning Electron Microscope:

SEM produces images by probing the specimen with a focused electron beam that is scanned across a rectangular area of the specimen. When the electron beam interacts with the specimen, it loses energy by a variety of mechanisms. The lost energy is converted into alternative forms such as heat, emission of low-energy secondary electrons and high-energy backscattered electrons, light emission (cathodoluminescence) or X-ray emission, all of which provide signals carrying information about the properties of the specimen surface, such as its topography and composition. The image displayed by an SEM maps the varying intensity of any of these signals into the image in a position corresponding to the position of the beam on the specimen when the signal was generated.

Advantage:

1. Although the image resolution of an SEM is lower than that of a TEM. But as the SEM images the surface of a sample rather than its interior, the electrons do not have to travel through the sample. This reduces the need for extensive sample preparation to thin the specimen to electron transparency.
2. The SEM is able to image bulk samples that can fit on its stage.
3. The SEM also has a great depth of field, and so can produce images that are good representations of the three-dimensional surface shape of the sample.
4. Another advantage of SEMs comes with environmental scanning electron microscopes (ESEM) that can produce images of good quality and resolution with hydrated samples or in low, rather than high, vacuum or under chamber gases. This facilitates imaging unfixed biological samples that are unstable in the high vacuum of conventional electron microscopes.

Atomic force microscopy (AFM)

Atomic force microscopy (AFM) is a powerful technique that enables the imaging of almost any type of surface, including polymers, ceramics, composites, glass and biological samples. AFM is used to measure and localize many different forces, including adhesion strength, magnetic forces and mechanical properties. AFM consists of a sharp tip that is approximately 10 to 20 nm in diameter, which is attached to a cantilever. AFM tips and cantilevers are micro-fabricated from Si or Si 3N4. The tip moves in response to tip–surface interactions, and this movement is measured by focusing a laser beam with a photodiode.

An AFM is operated in two basic modes, such as contact and tapping modes. In the contact mode, the AFM tip is in continuous contact with the surface. In contrast, in the tapping mode, the AFM cantilever is vibrated above the sample surface such that the tip is only in intermittent contact with the surface. This process helps to reduce shear forces associated with the tip movement. The tapping mode is the recommended mode that is commonly used for AFM imaging. The contact mode is only used for specific applications, such as force curve measurements. The AFM is used to image and manipulate atoms and structures on a variety of surfaces. The atom at the apex of the tip 'senses' individual atoms on the underlying surface when it forms incipient chemical bonds with each atom. Because these chemical interactions delicately alter the tip's vibration frequency, they can be detected and mapped.

Unlike the electron microscope, which provides a 2-D projection or a 2-D image of a sample, AFM provides a true 3-D surface profile. In addition, samples viewed by AFM do not require any special treatments (i.e. metal/carbon coatings) that would irreversibly change or damage the sample. Whereas an electron microscope requires an expensive vacuum environment for proper

operation, most AFM modes can work perfectly well in ambient air. In principle, AFM can provide higher resolution than SEM. It has been demonstrated that AFM can provide true atomic resolution in ultra-high vacuum (UHV) and, more recently, in liquid environments. High resolution AFM is comparable in resolution to scanning tunnelling microscopy and TEM. A disadvantage of the AFM technique compared to the SEM is the image size. The SEM can image an area in the order of millimetres by millimetres with a depth of field in the order of millimetres. The AFM can only image a maximum height in the order of micrometres and a maximum scanning area of approximately 150 by 150 micrometres.

In the case of clay-containing polymer nanocomposites, AFM has been used to image the surface of clay particles dispersed in a polymer matrix. For example, Yalcin and Cakmak used AFM to image the surface morphology of the intercalated silicate layers dispersed in Nano composite films.8 recently, researchers have also been using AFM to investigate the effect of incorporating Nano clay particles on the morphology and crystal growth behaviour of immiscible polymer blends.

UNIT-V

Sterilization

Physical Method to control microbial growth:

The control methods can be broadly divided into two categories physical and chemical methods

Physical method can be listed as follows

A. Heat: Moist and Dry Heat
B. Filtration
C. Low Temperature: Refrigeration, Deep freezing, Lyophilization
D. Desiccation
E. Osmotic pressure
F. Radiation: Ionizing and Non-Ionizing

A. Heat:

Heat is frequently used to eliminate microorganisms. Moist heat kills microbes by denaturing proteins (enzymes). Dry heat kills organisms by oxidation. For sterilization one must consider the type of heat, and most importantly, the time of application and temperature to ensure destruction of all microorganisms. Endospores of bacteria are considered the most thermoduric of all cells so their destruction guarantees sterility.

Thermal Death Point (TDP) is the lowest temperature at which all the microbes in a liquid culture will be killed in 10 minutes. Thermal Death Time (TDT) is the length of time required to kill all bacterial in a liquid culture at a given temperature. Decimal Reduction Time (DRT) is the length of time required to kill 90% of a bacterial population at a given temperature; D value. Z value is an increase in temperature required to reduce D by 1/10. F value is time in minutes at a specific temperature needed to kill a population of cells or spores.

Moist heat

Moist heat is thought to kill microorganisms by causing denaturation of essential proteins. Death rate is directly proportional to the concentration of microorganisms at any given time. Increasing the temperature decreases TDT, and lowering the temperature increases TDT. Processes conducted at high temperatures for short periods of time are preferred over lower temperatures for longer times.

Environmental conditions also influence TDT. Increased heat causes increased toxicity of metabolic products and toxins. TDT decreases with pronounced acidic or basic pH. However, fats and oils slow heat penetration and increase TDT. It must be remembered that thermal death times are not precise values; they measure the effectiveness and rapidity of a sterilization process. Autoclaving 121°C/15 psi for 15 minutes exceeds the thermal death time for most organisms except some extraordinary spore formers.

Common Examples of methods based on moist heat are:

a) Boiling

It involves heating at 100oC for 30 minutes. This method kills everything except some endospores. To kill endospores, and therefore sterilize a solution, very long (> 6 hours) boiling or intermittent boiling is required.

b) Autoclaving

Autoclaving is the most effective and most efficient means of sterilization. All autoclaves operate on a time/temperature relationship. These two variables are extremely important. Higher temperatures ensure more rapid killing. The usual standard temperature/pressure employed is 121ºC/15 psi for 15 minutes). Longer times are needed for larger loads, large volumes of liquid, and more dense materials.

Autoclaving is ideal for sterilizing biohazardous waste, surgical dressings, glassware, many type of microbiologic media, liquids, and many other things. However, certain items, such as plastics and certain medical instruments (e.g. fibre-optic endoscopes), cannot withstand autoclaving and should be sterilized with chemical or gas sterilants. When proper conditions and time are employed, no living organisms will survive a trip through an autoclave. The autoclave is a large pressure cooker; it operates by using steam under pressure as the sterilizing agent. High pressures enable steam to reach high temperatures, thus increasing its heat content and killing power. Most of the heating power of steam comes from its latent heat of vaporization. This is the amount of heat required to convert boiling water to steam. This amount of heat is large compared to that required to make water hot.

For example, it takes 80 calories to make 1 litre of water boil, but 540 calories to convert that boiling water to steam. Therefore, steam at 100ºC has almost seven times more heat than boiling water.

c) Pasteurization:

This heat treatment developed by famous microbiologist Louis Pasteur is used to destroy mostly pathogenic bacteria present in liquid medium e.g. milk and wine. Pasteurization is the use

of mild heat to reduce the number of microorganisms in a product or food. In the case of pasteurization of milk (Fig. 19.4), the time and temperature depend on killing potential pathogens that are transmitted in milk, i.e. staphylococci, streptococci, Brucella abortus and Mycobacterium tuberculosis. But pasteurization kills many spoilage organisms, as well, and therefore increases the shelf life of milk especially at refrigeration temperatures (2°C).,Milk is usually pasteurized by heating, typically at 63°C for 30 min (batch method) or at 71°C for 15 s (flash method), to kill bacteria and extend the milk's usable life. The process kills pathogens but leaves relatively benign microorganisms that can sour improperly stored milk. Various time-temperature combinations used for pasteurization are given in Table (19.3).

Dry heat

a) Hot air oven

Basically in the cooking oven the rules of relating time and temperature apply, but dry heat is not as effective as moist heat (i.e. higher temperatures are needed for longer periods of time). For example 160°/2h or 170°/1h is necessary for sterilization. The dry heat oven is used for glassware, metal, and objects that won't melt; used on substances that would be damaged by moist heat sterilization e.g. gauzes, dressings or powders.

b)Incineration

Burns organisms and physically destroys them (Fig. 19.5b). Used for needles, inoculating wires, glassware, etc. and objects not destroyed in the incineration process.

B. Filtration

Filtration is the passage of a liquid or gas through a screen like material with pores small enough to retain microorganisms. A vacuum that is created in the receiving flask aids gravity in pulling the liquid through the filter. Some operating theaters occupied by burn patients receive filtered air. High efficiency particulate air (HEPA) filter remove almost all microorganisms larger than 0.3 µm in diameters. Filtration is especially important for sterilization of solutions which would be denatured by heat (e.g. antibiotics, injectable drugs, amino acids, vitamins, etc.).

C. Low temperature

Low temperature (refrigeration and freezing): Most organisms grow very little or not at all at 0oC. Perishable foods are stored at low temperatures to slow rate of growth and consequent spoilage (e.g. milk). Low temperatures are not bactericidal. Psychrotrophs, rather than true psychrophiles, are the usual cause of food spoilage in refrigerated foods. Although a few microbes will grow in supercooled solutions as low as minus 20°C, most foods are preserved against microbial growth in the household freezer. The effectiveness of low temperatures depends on the particular microorganism and the intensity of the application. Most microorganisms do not reproduce at ordinary refrigerator temperatures (0-7°C); bacteriostatic.

Many microbes survive (but do not grow) at the sub-zero temperatures used to store foods.

D. Freeze drying

Microbes are placed in a suspending medium and frozen quickly at temperatures between -52 and 95°C. Water is removed by vacuum (sublimation) lyophilization. Powder-like product can be reconstituted to bring culture back to viable conditions.

E. Desiccation

Drying (removal of H2O): Most microorganisms cannot grow at reduced water activity (aw < 0.90). Drying is often used to preserve foods (e.g. fruits, grains, etc.). Methods involve removal of water from product by heat, evaporation, freeze-drying, and addition of salt or sugar.

F. Osmotic Pressure:

The use of high concentrations of salts and sugars in foods is used to increase the osmotic pressure and create a hypertonic environment.

Plasmolysis: As water leaves the cell, plasma membrane shrinks away from cell wall. Cell may not die, but usually stops growing.

Yeasts and molds: More resistant to high osmotic pressures. Staphylococci spp. that live on skin are fairly resistant to high osmotic pressure.

G. Irradiation

Irradiation (UV, X-ray, Gamma radiation): The effects of radiation depend on its wavelength, intensity, and duration. Ionizing radiation (Gamma rays, X-rays, and high-energy electron beams) has a high degree of penetration and exerts its effect primarily by ionizing water and forming highly reactive hydroxyl radicals. Ultraviolet (UV) radiation, a form of non-ionizing radiation, has a low degree of penetration and causes cell damage by making thymine dimers in DNA that interfere with DNA replication (Fig. 19.8). The most effective germicidal wavelength is 260 nm.

Microwave radiation

Wavelength ranges from 1 mm to 1 m. Heat is absorbed by water molecules. It may kill vegetative cells in moist foods. Bacterial endospores, which do not contain water, are not damaged by microwave radiation. Solid foods are unevenly penetrated by microwaves.

Gamma radiation and electron beam radiation

These radiations are formed of ionizing radiation used primarily in the health care industry. Gamma rays, emitted from cobalt-60, are similar in many ways to microwaves and x-rays. Gamma rays delivered during sterilization break chemical bonds by interacting with the electrons of atomic

constituents. Gamma rays are highly effective in killing microorganisms and do not leave residues or have sufficient energy to impart radioactivity.

Electron beam (e-beam) radiation, a form of ionizing energy, is generally characterized by low penetration and high-dose rates. E-beam irradiation is similar to gamma radiation in that it alters various chemical and molecular bonds on contact. Beams produced for e-beam sterilization are concentrated, highly-charged streams of electrons generated by the acceleration and conversion of electricity. E-beam and Gamma radiation are for sterilization of items ranging from syringes to cardiothoracic devices.

7. CONTROL OF MICROBIAL GROWTH BY CHEMICAL METHODS

Introduction

Chemical agents are the disinfectants that kill microorganisms, but not necessarily their spores, but are not safe for application to living tissues; they are used on inanimate objects such as tables, floors, utensils, etc. e.g. hypo chlorites, chlorine compounds, lye, copper sulphate, quaternary ammonium compounds, and formaldehyde and phenolic compounds

Phenol and phenolics

Phenolics exert their action by injuring the lipid-containing plasma membrane which results in leakage of cellular contents. Mycobacteria are susceptible to phenolics due to their rich lipid content e.g. Cresols (O-phenyl phenol, main ingredient in Lysol), bisphenols (Hexachlorophene, used in pHisoHex, effective against Gram positive cocci), triclosan (soap, toothpaste, plastics kitchenware; Gram positive and fungi).

Biguanides

Chlorohexidine damages plasma membranes of vegetative cells and is broad spectrum. These are commonly used for surgical hand scrubs. These are effective against most vegetative bacteria and fungi. Mycobacteria, endospores, and protozoan cysts are not affected.

Halogens

Some halogens (iodine and chlorine) are used alone or as components of inorganic or organic solutions. Iodine may combine with certain amino acids to inactivate enzymes and other cellular proteins. Iodine is available as a tincture (in solution with alcohol) or as an iodophor (combined with an organic molecule) like in Betadine. The germicidal action of chlorine is based on the formation of hypochlorous acid when chlorine is added to water. It is an excellent oxidizing agent. Chlorine is used as a disinfectant in gaseous form (Cl_2) or in the form of a compound, such as calcium hypochlorite, sodium hypochlorite, sodium dichloroisocyanurate, and chloramines.

Alcohol

Alcohols exert their action by denaturing proteins and dissolving lipids. In tinctures, they enhance the effectiveness of other antimicrobial agents. Aqueous ethanol (60-90%) and isopropanol are used as disinfectants. Not effective against spores or non-enveloped viruses.

Heavy metals and their compounds

Silver, mercury, copper, and zinc are used as germicidal. They exert their antimicrobial action through oligodynamic action. When heavy metal ions combine with sulfhydryl (-SH) groups, proteins are denatured. Examples are 1% Silver nitrate solution, mercuric chloride, copper sulphate (algicide).

Surface-active agents-soaps and acid anionic detergents

The agents decrease the surface tension among molecules of a liquid; soaps and detergents are examples. Soaps have limited germicidal action but assist in the removal of microorganisms through scrubbing. Acid-anionic detergents are used to clean dairy equipment.

Quaternary Ammonium Compounds: These are cationic detergents attached to NH4+. By disrupting the plasma membranes, they allow cytoplasmic constituents to leak out of the cell Quats are most effective against Gram-positive bacteria. They do not kill endospores or mycobacteria. Examples include Zephiran (benzalkonium chloride) and Cepacol (cetylpyridinium chloride). Pseudomonads are highly resistant, can even live in quats.

Organic acids and derivatives: This class of organic compounds is commonly used as food preservatives. These are effective mostly against mold as they interfere with mold metabolism or the integrity of the plasma membrane.

Nitrates

It can be found in some cheeses, adds sulphite, maintains pink colour in cured meats and prevents botulism in canned foods. Can cause adverse reactions in children, and potentially carcinogenic.

Sulphur dioxide and sulphites

These are used as preservatives and to prevent browning in alcoholic beverages, fruit juices, soft drinks, dried fruits and vegetables. Sulphites prevent yeast growth and also retard bacterial growth in wine. Sulphites may cause asthma and hyperactivity. They also destroy vitamins.

Benzoic acid and sodium benzoate: These are used to preserve oyster sauce, fish sauce, ketchup, non-alcoholic beverages, fruit juices, margarine, salads, confections, baked goods, cheeses, jams and pickled products. They have also been found to cause hyperactivity.

Propionic acid and propionates: These are used in bread, chocolate products, and cheese for lasting freshness.

Sorbic acid and sorbates: It prevents mold formation in cheese and flour confectioneries

Aldehydes

Aldehydes such as formaldehyde and glutaraldehyde (Gidex) exert their antimicrobial effect by inactivating proteins. They are among the most effective chemical disinfectants.

Gaseous chemosterilants: This class of chemosterilants includes chemicals that sterilize in a closed chamber. Chemicals used for sterilization include the gases ethylene oxide and formaldehyde, and liquids such as glutaraldehyde. Ozone, hydrogen peroxide and peracetic acid are also examples of chemical sterilization techniques are based on oxidative capabilities of the chemical.

Ethylene oxide (ETO)

It is the most commonly used form of chemical sterilization. Due to its low boiling point of 10.4°C at atmospheric pressure, ETO behaves as a gas at room temperature. ETO chemically reacts with amino acids, proteins, and DNA to prevent microbial reproduction. The sterilization process is carried out in a specialized gas chamber. After sterilization, products are transferred to an aeration cell, where they remain until the gas disperses and the product is safe to handle. ETO is used for cellulose and plastics irradiation, usually in hermetically sealed packages. Ethylene oxide can be used with a wide range of plastics (e.g. petri dishes, pipettes, syringes, medical devices, etc.) and other materials without affecting their integrity).

Ozone sterilization has been recently approved for use in the U.S. It uses oxygen that is subjected to an intense electrical field that separates oxygen molecules into atomic oxygen, which then combines with other oxygen molecules to form ozone. Ozone is used as a disinfectant for water and food. It is used in both gas and liquid forms as an antimicrobial agent in the treatment, storage and processing of foods, including meat, poultry and eggs. Many municipalities use ozone technology to purify their water and sewage.

Low temperature gas plasma (LTGP)

It is used as an alternative to ethylene oxide. It uses a small amount of liquid hydrogen peroxide (H_2O_2), which is energized with radio frequency waves into gas plasma. This leads to the generation of free radicals and other chemical species, which destroy organisms.