MARUDHAR KESARI JAIN COLLEGEFOR WOMEN

VANIYAMBADI

DEPARTMENT OF BIOTECHNOLOGY

MICROBIOLOGY

II B.Sc BIOTECHNOLOGY **SUBJECT CODE FBT31**

MS. S ANU PRIYA

UNIT -1

History of microbiology, Classification of microorganisms (kingdom protista, prokaryotic and eukaryotic microorganisms) Five kingdom concept of classification, Archaebacteria, eubacteria and eukaryotes.

Always trust a microbiologist

Because they have the best

Chance of predictine when the world will end.

-teddie O. Rahube

INTRODUCTION

- Microorganisms are the dominant life forms on earth, are found in almost every conceivable environment, and are essential to sustaining life on this planet.
- The term microbiology was given by French chemist Louis Pasteur (1822-95).

- Microbiology is said to have its roots in the great expansion and development of the biological sciences that took place after 1850. The term microbe was first used by Sedillot (1878).

There are five basic groups of microorganisms:

- **Bacteria** are typically unicellular, microscopic, prokaryotic organisms that reproduce by binary fission.
- **Fungi (**yeasts and molds) are typically unicellular, microscopic, eukaryotic fungi that reproduce asexually by budding. Molds are typically filamentous, eukaryotic fungi that reproduce by producing asexual reproductive spores.
- **Viruses** are typically submicroscopic, acellular infectious particles that can only replicate inside a living host cell. The vast majority of viruses possess either DNA or RNA, but not both.
- **Protozoa** are typically unicellular, microscopic, eukaryotic organisms that lack a cell wall.
- **Algae** are typically eukaryotic microorganisms that carry out photosynthesis.

HISTORY OF MICROBIOLOGY

Microbiology - The science that studies very small living things.Usually requires a magnification tool – the microscope . Some organisms are large though Helminths – worms

Sub groups of Microbes we will study

- Bacteria
- Archaea
- Fungi
- Protozoans
- Algae
- Viruses
- Multicellular animal parasites – Helminths
- Various disciplines of study within microbiology:
- Bacteriology, Mycology, Parisitology, Immunology,
- Epidemiology,
- Biotechnology

- Virology
- Environmental Microbiology

HISTORICAL REVIEW OF THE SCIENCE OF MICROBIOLOGY

Robert Hook – 1665 – Englishman, used a primitive compound (two magnifying lenses) microscope, reported that life's smallest units were little boxes – **Cells**, his work started the process of the development of the **Cell theory** of life.

Antoni Van Leeuwenhoek –1673 - probably the first person to observe living cells with a simple microscope, amateur scientist, ground his own lenses and described what we know today as bacteria – rod shaped , spiral shaped , etc. "animalcules"

- Actually he was a Dutch linen merchant but spent much of his spare time constructing simple microscopes composed of double convex lenses held between two silver plates. He constructed over 250 small powerful microscopes that could magnify around 50-300 times.
- Leeuwenhoek was the first person to produce precise and correct descriptions of bacteria and protozoa using a microscope he made himself. Because of this extraordinary contribution to microbiology, he is considered as the "Father of microbiology".
- Leeuwenhoek is also considered to be the father of bacteriology and protozoology (protistology).

The main aspects were to solve the controversy over spontaneous generation which includes experimentations mainly of **Francesco Redi, John Needham, Lazzaro Spallanzani** and **Nicolas Appert** etc and to know the disease transmission whicmainly includes the work of **Ignaz Semmelweis** and **John Snow**.

SPONTANEOUS GENERATION

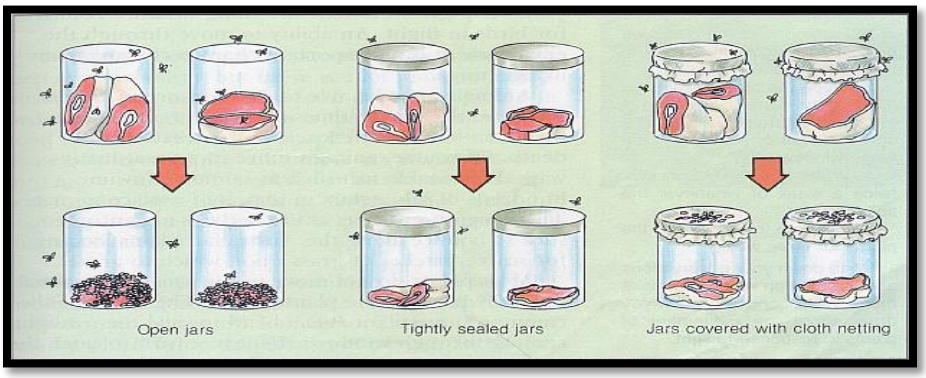
The hypothetical process by which living organisms develop from nonliving matter; also, the archaic theory that utilized this process to explain the origin of life. Many believed in spontaneous generation because it explained such occurrences as the appearance of maggots on decaying meat.

JOHN NEEDHAM

Contributions and Achievements:
Needham established from **his observations that micro-organisms do not grow from eggs and proposed a theory of spontaneous generation whereby living organisms develop from non-living matter at the microscopic level**. He carried out microscopic observations with the comte de Buffon in 1748.

FRANCESCO REDI (1626-1697):

- The ancient belief in spontaneous generation was first of all challenged by Redi, an Italian physician, who carried out a series of experiments on decaying meat and its ability to produce maggots spontaneously.
- Redi went on to demonstrate that **dead maggots or flies would not generate new flies when placed on rotting meat in a sealed jar**, whereas live maggots or flies would. This disproved both the existence of some essential component in once-living organisms, and the necessity of fresh air to generate life.

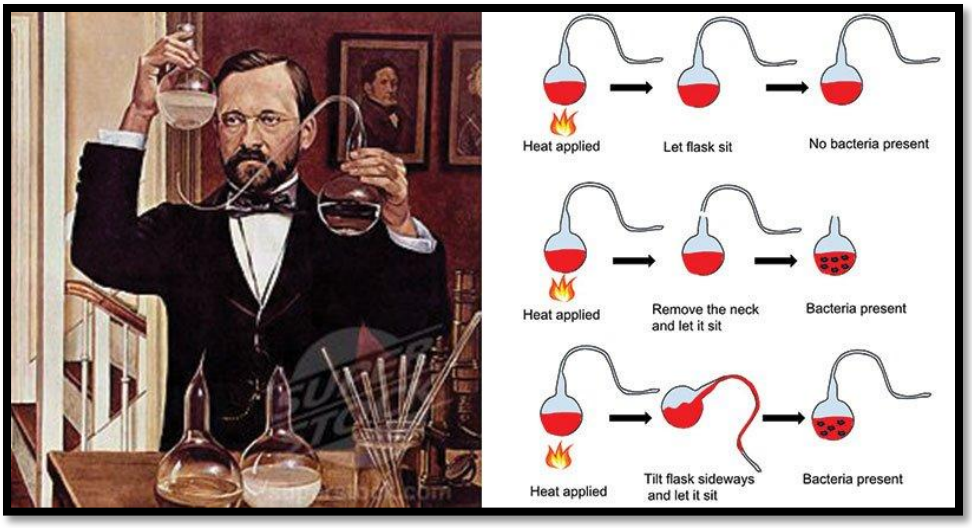


EDWARD JENNER

- Edward jenner is credited with first **vaccine** – in epidemics of smallpox during the late 1700's he observed that milk maids didn't get the disease, cattle had a similar disease – **cowpox**, milk maids had cow pox lesions, but not small pox, he purposefully took scrapings from cowpox blister and scraped a 8 year old volunteer.
- With the material – child got mild illness but not small pox,
- **Vaccination** comes from Latin word "vacca" meaning cow. Jenner laid the foundation for Pasteur's later work with other vaccinations.
- On may 14th,1796 he devised a performed a vaccination against the small pox by transferring material from cowpox pustle on the hand of a milkmaid to the hand of a small boy named James Phipps.
- Six weeks later the boy was inoculated with smallpox and he failed to develop the disease.
- He described about rheumatic heart disease and purified important medicines.

LOUIS PASTEUR

- French sceintist that dealt the death blow to the spontaneous generation theory.
- Louis Pasteur designed a procedure to **test whether sterile nutrient broth could spontaneously generate microbial life**. To do this, he set up two experiments. In both, Pasteur added nutrient broth to flasks, bent the necks of the flasks into S shapes, and then boiled the broth to kill any existing microbes.
- He devised the ingenious curved necked flasks that prevented contaminated air from reaching boiled beef broth – the broth remained uncontaminated even though exposed to the air
- He was very lucky – no **endopores** present, or it would have failed (resitant to boiling)



1. He developed process we call Pasteuriztion – he heated wine to kill contaminating microbes – cured sick wine (today we heat treatment to kill pathogens in milk also)

2. He proved that fermentation was caused by a microbe – yeast

3. He developed vaccines for rabies and anthrax. Vaccines led to immunity to diseases that routinely killed many people, used to help people long before they understood how they even worked (science of Immunology)

4. He began the revolution in science that led to the **Golden Age of**

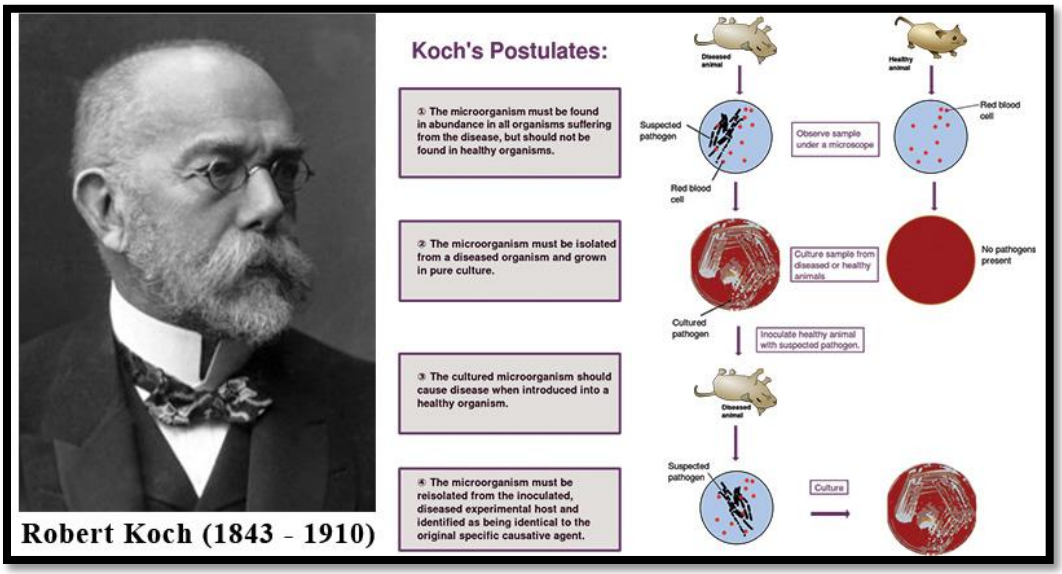
ROBERT KOCH

Developed **Koch's postulates** – important technique for determining the actual microbial cause agent of a disease – more later, German, contemporary of Pasteur, several very important contributions

1. He discovered the tuberculosis bug (tubercle bacillus, Mycobacterium tuberculosis)

2. He discovered the cause of anthrax (Bacillus anthracis) – from blood of dead cattle, cultured bacteria in pure culture, injected bacteria in live cattle and they died, then again cultured the bacteria in pure culture.

This led to the establishment of a procedure for determining microbial cause of **Koch's and Pasteur's work helped establish the "Germ Theory of Disease" - that microorganisms cause disease (in people, animals, and even plants**)



WHAT IS MEANT BY THE GERM THEORY OF DISEASE?

Germ theory, in medicine, the theory **that certain diseases are caused by the invasion of the body by microorganisms**, organisms too small to be seen except through a microscope.

PAUL EHRLICH

- German biochemist Paul Ehrlich (1854–1915) **developed a chemical theory to explain the body's immune response** and did important work in chemotherapy, coining the term magic bullet. Ehrlich received the Nobel Prize in Physiology or Medicine in 1908.
- Ehrlich and Hata tested **606 over and over on mice, guinea pigs, and then rabbits with syphilis**. They achieved complete cures within three weeks, with no dead animals. In 1910 the drug was released, called Salvarsan, or sometimes just 606. It was an almost immediate success and was sold all over the world.
- **Ehrlich's** research career began with selective cell staining with dyes, which allowed him to identify mast cells and the different types of granulocytes.

Such studies led him to formulate the concept of molecules that specifically bind to cell receptors; like a key that can only open the lock it was made for.

JOSEPH LISTER

He was the **first person to isolate bacteria in pure culture (Bacillus lactis) using liquid cultures** containing either Pasteur's solution of turnip infusion and a special syringe to dilute the inoculum and so can be considered a co-founder of medical microbiology with Koch, who later isolated bacteria on solid media.

1. First antiseptic use in surgery, chemicals used as agents on tissue before surgery (tissue treated with an antimicrobial agent – antiseptic, betadine) disinfectants are chemicals, used on a surface

2. Also proved that microbes cause surgical infections

JOHN TYNDALL

- During the 1870s, John Tyndall and a number of other British scientists observed that **Pénicillium sp. inhibited bacterial growth**.
- Tyndall concluded that fungi, growing in various meat and vegetable infusions killed bacteria by excluding oxygen. Some of Tyndall's experiments were repeated here.

CLASSIFICATION OF MICROORGANISM

- Taxonmoy: Science of classifying organisms. Provides universal names for organisms. Taxonomic categories: Taxon / Taxa
- Phylogeny or Systematics: Evolutionary history of group of organisms.
- Taxonomic hierarchy shows phylogenetic (evolutionary),relationships among organisms.
- 1969: Living organisms divided into five kingdoms
- 1978: Two types of prokaryotic cells found. Prokaryotic relationships determined by rRNA sequencing

There are various types of microorganisms like bacteria, fungi, viruses, algae, archaea, and protozoa.

BACTERIA:

- Bacteria are the most common microorganism that we hear most in our daily lives Isn't it! They are a member of prokaryotes.
- They do not have a nucleus inside the cell and do not contain organelles. So, mainly they are specialised cellular organs.
- Bacteria consist of two classes namely Gram-positive bacteria which consists thicker cell wall and another is Gram-negatives which have a thinner layer that is sandwiched between an inner and outer membrane.

FUNGI:

- They are eukaryotes that have defined nucleus and organelles.
- When fungi achieved a certain level of growth, they are visible via eye like a mould on bread.

VIRUSES:

- According to various experts viruses are not living organisms. They consist of nucleic acid that is DNA or RNA and a protein coat. Basically, a virus requires a host cell for replication. The virus enters human beings and enters the cell where they replicate.
- It depends upon the immune system of the body. If the immune system is weak then the virus may affect the human being and may cause diseases like a common cold, influenza, etc. In fact, some viruses may cause permanent and irreversible damage to the cells of human beings for example HIV.

***ALGAE*:**

- According to some definitions algae consists of prokaryotes and eukaryotes both.
- Algae, unlike other microorganisms, are photosynthesisers and are typically found in marine environments. Prokaryotic algae are known as Cyanobacteria or blue-green algae.
- But some say that algae are eukaryotes only that is they are small aquatic plants.

KINGDOM: PROTISTA

DEFINE KINGDOM PROTISTA

- Protista is aquatic small unicellular, i.e., single-celled, eukaryotic microorganisms with a well-defined nucleus.

HISTORY OF KINGDOM PROTISTA

- Coming to the history of this kingdom, according to the evolution, this kingdom Protista acts as a connecting link between the prokaryotic kingdom Monera and other multicellular kingdoms like Fungi, Animalia and Plantae.
- The term 'Protista' was given by German biologist Ernst Haeckel in the year 1866. The kingdom Protista was included in the third kingdom classification.

CHARACTERISTICS OF KINGDOM PROTISTA

The group Protista shows the following characteristics in common:

- Protista are unicellular eukaryotic organisms.
- The members of the kingdom Monera are mostly aquatic in habitat.
- The cell wall is present in some protists and absent in some other forms.
- The eukaryotic cell of protists possesses a well-defined nucleus, and membrane-bound organelles also present.
- Nutrition may be autotrophic and heterotrophic.
- The organisms show locomotion with the help of cilia, flagella or pseudopodia.
- Reproduction is by both sexual and asexual reproduction.

KINGDOM PROTISTA FLOW CHART

- The Kingdom Protista includes three broad groups, as shown in the following flow chart:

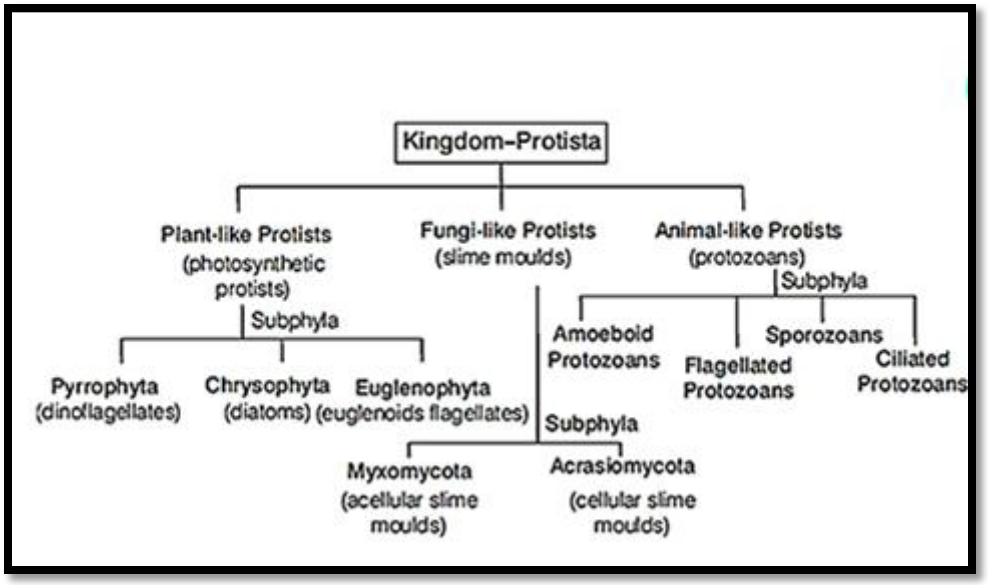


Fig: A Flowchart of the Kingdom Protista

CLASSIFICATION OF KINGDOM PROTISTA

The kingdom Protista is divided into three groups, namely, Plant-like Protists, Fungi-like Protists and Animal-like Protists.

PLANT-LIKEPROTISTS

These are organisms that show plant-like characteristics and are also photosynthetic organisms. It is of three sub-types namely, Dinoflagellates, Chrysophytes and Euglenoids.

Dinoflagellates

The group of around 1000 species of photosynthetic protists belongs to the division – **Pyrrophyta** and class – **Dinophyceae**.
The members show the following characteristics:

- These are photosynthetic, i.e., autotrophic in nutrition.
- These are motile, biflagellate and mostly marine forms.
- They contain green, yellow, brown, red or blue pigments.
- These form the important components of **phytoplankton**.

- Their macronuclei possess condensed chromosomes, even in interphase, called **mesokaryon.**
- Some dinoflagellates emit **light** and glow in the dark. This refers to the phenomenon of **bioluminescence**.
- They also emit toxins, and toxins released by them make the sea appear red, and they even kill marine organisms. This red tide is also caused by the colouration of the dinoflagellates, and this phenomenon of red tide is seen during the rapid multiplication of the organism.
- Reproduction is by both asexual and sexual methods.
- **Examples:** *Gonyaulax*, *Noctiluca*, etc.

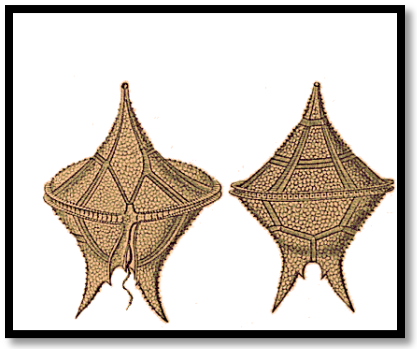


Fig: Dinoflagellates

CHRYSOPHYTES

These are called jewels of the plant world.

The members show the following characteristics:

- These are unicellular free-floating fresh or marine water forms.
- Most of them are photosynthetic, and their cell wall is made up of **silica** and **pectin**.
- Reproduction is by both sexual and asexual methods.
- The accumulation of a large amount of cell wall deposit of Diatoms is referred to as **diatomaceous earth** (can be used as fuel after mining).
- In Diatoms, the cell wall form two thin overlapping shells, that fit together as in a soapbox.
- **Example:** Diatoms, Desmids, golden algae, etc.

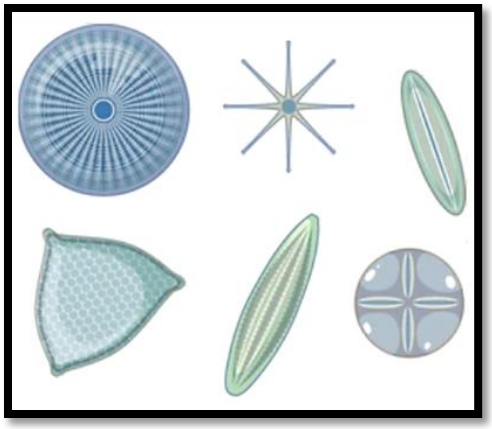


Fig: Diatoms With Different Shapes

EUGLENOIDS

These are unicellular and share the characteristics of both plants and animals.

The members show the following characteristics:

- These are green, autotrophic in nutrition (plant character).
- These are *Euglena* like unicellular flagellates (animal character) found mostly in stagnant freshwater.
- They have two types of flagella – **Long Whiplash** and **Short Tinsel**.
- Instead of a cell wall, they have a protein-rich layer called **pellicle**, which makes their body flexible.
- The food is stored in proteinaceous granules known as **pyrenoids**.
- Reproduction is only by the asexual method.
- Photosynthetic euglenoids behave like heterotrophs in the dark, this mode of nutrition is known as **mixotrophic**.
- **Example**: *Euglena* – The chief member of this group and regarded as the connecting link between animals and plants.

Fig: *Euglena*

FUNGI-LIKE PROTISTS (SLIME MOULDS)

They possess the characters of both animals and fungi, therefore, combinedly called fungus-animals.

The members show the following characteristics:

- They occur in moist terrestrial habitats and are seen moving along the decaying twigs and leaves.
- They reproduce by both sexual and asexual methods.
- These show saprophytic nutrition.
- Under suitable conditions, they form *Plasmodium*. On the basis of occurrence of *Plasmodium*, these are of two types:
  a. Acellular/Plasmodial slime moulds, E.g., *Fuligo septica*, *Physarum*, etc.
  b. Cellular slime moulds, E.g., *Dictyostelium*, *Polysphondylium*, etc.

Fig: Slime moulds

ANIMAL-LIKE PROTISTS (PROTOZOANS)

These are the most primitive relatives of animals and these protozoans are heterotrophic organisms.
They are divided into four major groups, namely,

AMOEBOID PROTOZOANS

- They live in freshwater, moist soil and saltwater.
- They move with the help of pseudopodia, as in ***Amoeba***.

- Other members of this group are *Entamoeba histolytica* and *E. gingivalis* that causes various digestive and oral diseases or infections when engulfed through contaminated water.

Fig: *Amoeba*

FLAGELLATED PROTOZOANS

- These are either free-living or parasitic in nature.
- Chief members of this group are as follows:
  (a). ***Trypanosoma sp.*** **–** carried by the **tsetse fly** and causes **African sleeping sickness**.
  (b). ***Leishmania sp.*** **–** carried by **sand fly** and causes **kala-azar** or **dum-dum fever**.
  (c). ***Giardia sp.*** **–** causes **giardiasis**.
  (d). ***Trichomonas vaginalis*** **–** cause **leucorrhoea**.

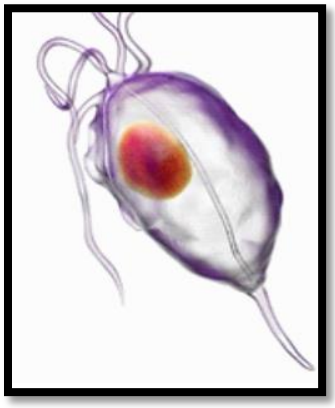


Fig: *Trichomonas vaginalis*

CILIATED PROTOZOANS

- They are aquatic, and locomotion is due to the presence of cilia.
- They show nuclear **dimorphism** (macro and micronucleus), E.g., *Paramecium*, etc.
  (a). **Macronucleus/Vegetative nucleus –** This helps in controlling the metabolic activities and growth.

(b). **Micronucleus/Reproductive nucleus –** This helps in controlling the reproduction process.

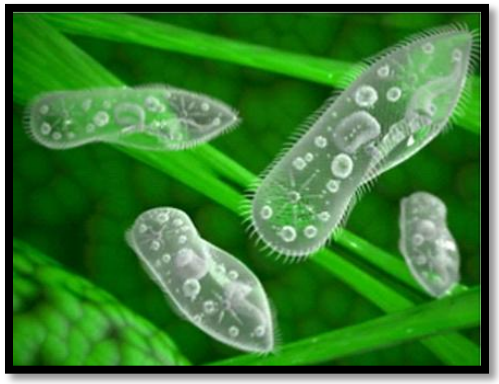


Fig: *Paramecium*

SPOROZOANS

- These Sporozoans processes an infectious, spore-like stage in their life cycle.
  All are endoparasites.
  These move with the help of the locomotory organs called cilia, flagella and pseudopodia.
  Example: *Plasmodium*, *Monocystis*, etc.

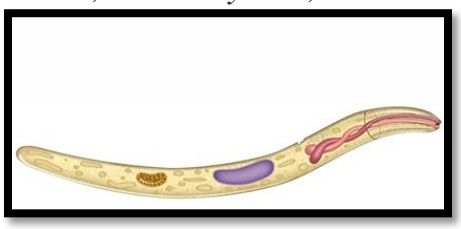


Fig: *Plasmodium*

ECONOMIC IMPORTANCE OF KINGDOM PROTISTA

The economic importance of the kingdom Protista are as follows:

- The protists play a very important role in the aquatic food chain.
- Certain types of seaweed may be consumed as food in some countries.
- Diatomaceous earth can be used as fuel after mining (Most of the oils and gasoline supply comes from diatoms fossil beds).
- Some of the protists may be used for the production of cosmetics, drugs and vitamins.

- Deposits of these diatoms' sheets used as car polishes, cleansers, paints and also used in toothpaste.
- Red algae contain algin that is used in the preparation of ice creams, cereals, candies, marshmallows, jams and jellies, chocolates, etc.
- Environmental indicators of pollution.

PROKARYOTIC AND EUKARYOTIC MICROORGANISMS

WHAT ARE PROKARYOTIC AND EUKARYOTIC MICROBES?

- **Prokaryotes are organisms that consist of a single prokaryotic cell**. Eukaryotic cells are found in plants, animals, fungi, and protists.
- They range from 10–100 μm in diameter, and their DNA is contained within a membrane-bound nucleus.
- Eukaryotes are organisms containing eukaryotic cells.

WHAT IS THE DIFFERENCE BETWEEN EUKARYOTIC MICROORGANISMS AND PROKARYOTIC MICROORGANISMS?

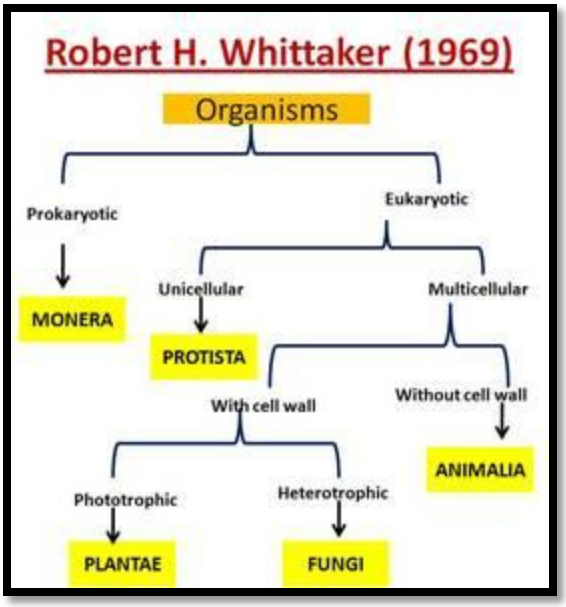
- Prokaryotes are organisms made up of cells that lack a cell nucleus or any membrane-encased organelles. Eukaryotes are organisms made up of cells that possess a membrane-bound nucleus that holds genetic material as well as membrane-bound organelles.

ARE MICROBES PROKARYOTIC OR EUKARYOTIC?

- Microorganisms are found in each of the three domains of life: Archaea, Bacteria, and Eukarya. Microbes within the domains Bacteria and Archaea are **all prokaryotes** (their cells lack a nucleus), whereas microbes in the domain Eukarya are eukaryotes (their cells have a nucleus).

FIVE KINGDOM CLASSIFICATION

Whitaker proposed that organisms should be broadly divided into kingdoms, based on certain characters like the structure of the cell, mode of nutrition, the source of nutrition, interrelationship, body organization, and reproduction. According to this system, there are five main kingdoms.



They are:

- Kingdom Monera
- Kingdom Protista
- Kingdom Fungi
- Kingdom Animalia
- Kingdom Plantae

KINGDOM MONERA

- ❖ These organisms are prokaryotic and unicellular. They do not have a well-defined nucleus and also lack cell organelles.

- ❖ Some organisms show the presence of cell wall while there are others without a cell wall.

- ❖ Consequently, some organisms are autotrophic and others are heterotrophic. Examples include *Bacteria, Cyanobacteria, and Mycoplasma.*

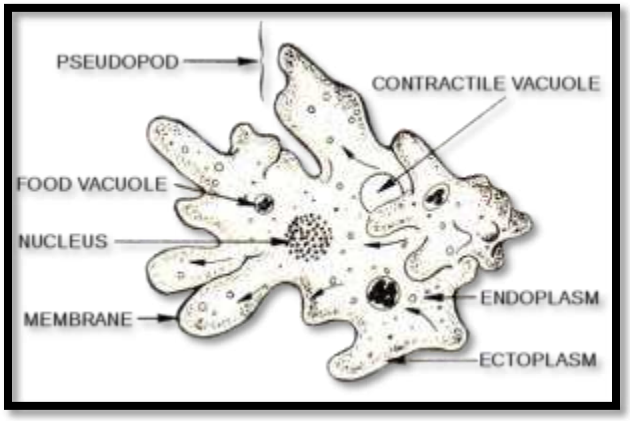
KINGDOM PROTISTA

- Organisms grouped under Kingdom Protista are all unicellular, but eukaryotic organisms.

- These are the simplest forms of eukaryotes that exhibit either autotrophic or heterotrophic mode of nutrition.

- Some organisms have appendages such as cilia or flagella or pseudopodia to move around.

- Some examples are *Diatoms, Protozoans like Amoeba, Paramoecium*



KINGDOM FUNGI

- Heterotrophic, Multicellular and Eukaryotic organisms are grouped under Kingdom Fungi.

- Their mode of nutrition is saprophytic as they use decaying organic matter as food. They have cell walls, which are made up of a substance called Chitin.

- Fungi also form a symbiotic association with some blue-green algae.

- *Yeast, Mushroom, Aspergillus* are examples of Fungi.

KINGDOM PLANTAE

- These are Eukaryotic, Multicellular organisms with a cell wall that is made up of cellulose.
- They are autotrophs and synthesize their own food through the process of photosynthesis. This kingdom includes all plants.
- Based on the body differentiation and presence or absence of specialized vascular tissue, Kingdom Plantae is divided into different divisions, namely Thallophyta, Bryophyta, Pteridophyta, Gymnosperms, and Angiosperms.
- Examples are *Spirogyra, Ferns, Pines, and Mango Plant* etc.

KINGDOM ANIMALIA

- This Kingdom includes organisms that are Multicellular, Eukaryotic, without the presence of cell wall.
- They have a heterotrophic mode of nutrition. They also exhibit great diversity.
- Some organisms are simple while others have a complex body with specialized tissue differentiation and body organs.
- The Animal Kingdom is divided into many phyla and classes. Some of the phyla are Porifera, Coelenterata, Arthropoda, Echinodermata, Chordata etc.
- Examples – *Hydra, Starfish, Earthworms, Monkeys, Birds* etc.

ARCHAEBACTERIA, EUBACTERIA AND EUKARYOTES

- Archaebacteria are one of the oldest living organisms (to be known) on Earth.
- They are classified as bacteria because many of their features resemble the bacteria when observed under a microscope.
- They belong to the kingdom Archaea and hence are named Archaebacteria.

- ❖ They share slightly common features with eukaryotes but are completely different from prokaryotes.

ARCHAEBACTERIA

- Both kingdoms of bacteria have species that are microscopic;
- Humans can't see them unless we stain them and then look at them under the microscope
- Archaebacteria (Live in harsh environment)

CHARACTERISTICS

- Have a cell membrane
- Have a cell wall
- Prokaryotic (no nucleus/organelles)
- Live in damp, watery places
- Reproduce by asexual fission

BASIC FACTS

- They live in extreme environments (like hot springs or salty lakes) and normal environments (like soil and ocean water).
- All are unicellular (each individual is only one cell).
- No peptidoglycan in their cell wall.
- Some have a flagella that aids in their locomotion.

3 MAIN TYPES

- ❖ Methanogens
- ❖ Thermo acido philes
- ❖ Halo philes

METHANOGENS

- They release methane (CH_4) as a waste product
- Many live in mud at the bottom of lakes and swamps because it lacks oxygen
- Some live in the intestinal tracts of animals to help break down food
- Others like to hang out in the stomach
- Your intestinal gas is a waste product caused by bacteria in the body breaking down the food you eat—that's why farts don't smell sweet!

THERMOACIDOPHILES

- Live in the dark
- Live without oxygen
- Like to live in superheated water with temperatures reaching 750 deg F
- Prefer environments that are very acidic (between pH of 1-3)
- Live in a chemical soup of hydrogen sulfide (H_2S) and other dissolved minerals (rotten egg smell)

Thermo = temperature

Acidophil = acid loving

HALOPHILES

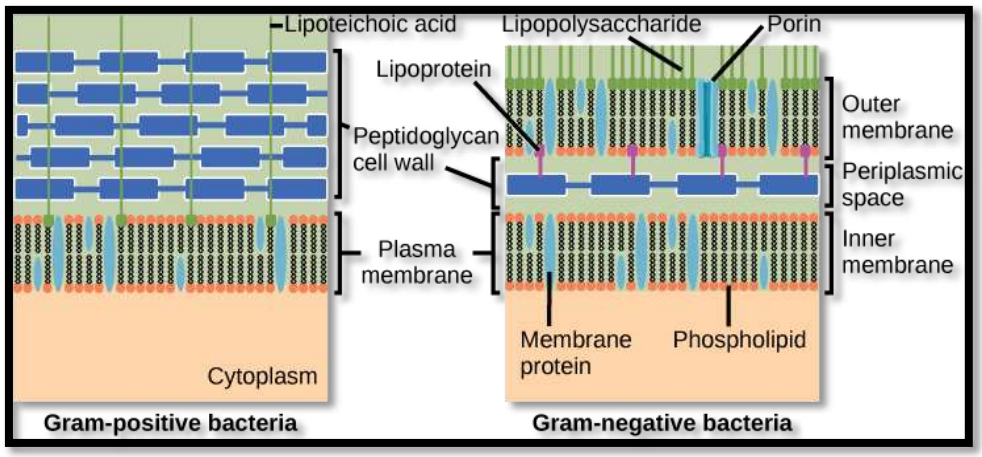
- Can live in water with salt concentrations exceeding 15%
- The ocean's concentration is roughly 4%
- Halo = salt

  phil = loving

EUBACTERIA

- "true bacteria"

- ❖ Eubacteria are a more complex domain of kingdom monera.
- ❖ They are found in most of the habitats on earth like soil, water and inside or outside of large organisms.
- ❖ Since eubacteria do not consist of membrane-bound organelles, almost all the metabolic reactions take place in the cytoplasm.
- ❖ Some eubacteria are involved in the nitrogen cycle as well.
- ❖ They also exhibit both parasitic and pathogenic effects on their host organisms.
- ❖ **Eubacteria** are prokaryotic microorganisms consisting of a single cell lacking a nucleus and containing DNA is a single circular chromosome.
- ❖ Eubacteria can be either gram-negative or gram-positive, they have economic, agricultural, and medical importance.
- ❖ They include *E. coli*, *Lactobacilli,* and *Azospirillum.*



- ❖ Depending on the cell wall thickness, eubacteria can be divided into two categories: gram positive and gram negative bacteria.
- ❖ The peptidoglycan layer of gram positive bacteria binds with the gram stain, giving positive results.
- ❖ The cell wall structure of gram negative bacteria is more complex than gram positive bacterial cell wall and incapable of binding with gram stain.

DIFFERENCE BETWEEN ARCHAEBACTERIA AND EUBACTERIA

ALTERNATIVE NAMES

- **Archaebacteria:**
  Archaebacteria are called ancient bacteria.
- **Eubacteria:**
  Eubacteria are called true bacteria.

SIZE

- **Archaebacteria:**
  Individual archaebacterium is 0.1-15 μm in diameter.
- **Eubacteria:**
  Individual eubacterium is 0.5-5 μm in diameter.

SHAPE

- **Archaebacteria:**
  Archaebacteria are spheres, rods, plates, spiral, flat or square-shaped.
- **Eubacteria:**
  Eubacteria are cocci, bacilli, vibrio, rods, filaments or spirochetes in shape.

COMPLEXITY

- **Archaebacteria:**
  Archaebacteria are simple in their organization.
- **Eubacteria:**
  Eubacteria are more complex than archaebacteria.

HABITAT

- **Archaebacteria:**
  Archaebacteria are found in extreme environments.
- **Eubacteria:**
  Eubacteria are found everywhere on earth.

CELL WALL

- **Archaebacteria:**
  Cell wall is composed of pseudo peptidoglycans.
- **Eubacteria:**
  Cell wall is composed of peptidoglycans with muramic acid.

MEMBRANE LIPIDS

- **Archaebacteria:**
  Membrane lipids of archaebacteria is ether-linked, branched, aliphatic chains, containing D-glycerol phosphate.
- **Eubacteria:**
  Membrane lipids of eubacteria are ester-linked, straight chains of fatty acids, containing L-glycerol phosphates.

RNA POLYMERASE

- **Archaebacteria:**
  RNA polymerase of archaebacteria consists of a complex subunit pattern, which is similar to eukaryotic RNA polymerase.
- **Eubacteria:**
  RNA polymerase of eubacteria consists of a simple subunit pattern.

TRANSFER RNA

- **Archaebacteria:**
  No thymine is present in the TψC arm of the tRNA, carrying methionine.
- **Eubacteria:**
  Thymine is present in most of the tRNA, carrying N-formyl methionine.

INTRONS

- **Archaebacteria:**
  Introns are present in archaebacteria.
- **Eubacteria:**
  Introns are absent in eubacteria.

GROWTH AND REPRODUCTION

- **Archaebacteria:**
  Asexual reproduction methods like binary fission, budding and fragmentation are used by archaebacteria during their reproduction.
- **Eubacteria:**
  Other than binary fission, budding and fragmentation, eubacteria are capable of producing spores in order to remain dormant during unfavorable conditions.

GLYCOLYSIS/KREB'S CYCLE

- **Archaebacteria:**
  Archaebacteria exhibit neither glycolysis nor Kreb's cycle.
- **Eubacteria:**
  Eubacteria exhibit both glycolysis and Kreb's cycle.

TYPES

- **Archaebacteria:**
  Archaebacteria are three types: methanogens, halophiles and thermophiles.
- **Eubacteria:**
  Eubacteria are two types: gram positive and gram negative.

EXAMPLES

- **Archaebacteria:**
  Halobacterium, Lokiarchaeum, Thermoproteus, Pyrobaculum, Thermoplasma and *Ferroplasma* are the examples of archaebacteria.
- **Eubacteria:**
  Mycobacteria, Bacillus, Sporohalobacter, Clostridium and *Anaerobacter* are the examples of eubacteria.

NUTRITION IN EUBACTERIA

1. Heterotrophic

- use food produced by other organisms

2. Saprotrophic

- feed on dead or decaying matter

3. Autotrophic

- make their own food

EUKARYOTES

- **Eukaryote** refers to any of the single-celled or multicellular organisms whose cell contains a distinct, membrane-bound nucleus.

- Organisms such as animals, plants, fungi, and protists are examples of eukaryotes because their cells are organized into compartmentalized structures called organelles, such as the nucleus.
- The presence of a distinct nucleus encased within membranes differentiates the eukaryotes from the prokaryotes.
- The eukaryotes are also known for having cytoplasmic organelles apart from nucleus, such as mitochondria, chloroplasts, and Golgi bodies.
- Eukaryotes often have unique flagella made of microtubules in a 9+2 arrangement.

EUKARYOTIC CELL

Parts of a typical animal cell:
(1) nucleolus, (2) nucleus, (3) ribosomes, (4) vesicle, (5) rough endoplasmic reticulum, (6) Golgi apparatus, (7) cytoskeleton, (8) smooth endoplasmic reticulum, (9) mitochondrion, (10) vacuole, (11) cytosol, i.e. the fluid that contains organelles, comprising the cytoplasm, (12) lysosome, (13) centrosome, (14) cell membrane.

EXAMPLES OF EUKARYOTES

All eukaryotes belong to Domain Eukaryota. Organisms belonging to this domain are animals, plants, fungi, and protists.

ANIMALS

Animals are eukaryotes that distinct from the other groups of eukaryotes by being heterotrophic, motile, and multicellular, a body organized into cells, tissues, organs, and systems, lacking cell walls and chloroplasts, and growing from a blastula during embryonic development.

PLANTS

Plants are photosynthetic eukaryotes. They have chlorophyll and other pigments that help in photosynthesis. They have a cell wall comprised mainly of cellulose. It

provides structural support. They are not as motile as the animals. Movements are limited but their growth is not. They are capable of unlimited growth through meristematic tissues. They lack the sense organs in animals. Nevertheless, they can sense certain stimuli and respond accordingly by tropisms.

FUNGI

Similar to plants, fungi have cell walls. However, the cell walls are made up chiefly of chitin (material in the exoskeleton of insects). Fungi lack chlorophyll and therefore are heterotrophic. Many of them are multicellular, forming hyphae and mycelium. Few species are unicellular. Examples of fungi are yeasts, rusts, stinkhorns, puffballs, truffles, molds, mildews, and mushrooms.

PROTISTS

Protists are unicellular eukaryotes. However, some species form filaments or colonies of the same species. They move around as they have locomotory organs, such as pseudopods, cilia, and flagella. Others lack these organs and therefore are non-motile.

Protists include the following:

(1) protozoa, the animal-like protists,

(2) algae, the plant-like protists, and

(3) slime molds and water molds, the fungus-like protists.

study hard ,

no matter if it seems impossible,

no matter if it takes time,

no matter if you have to up all night,

just remember that

the feeling of success is

the best thing in entire world.

MARUDHAR KESARI JAIN COLLEGE FOR WOMEN

VANIYAMBADI

DEPARTMENT OF BIOTECHNOLOGY

MICROBIOLOGY

II B.Sc BIOTECHNOLOGY **SUBJECT CODE: FBT31**

MS. S. ANU PRIYA

UNIT -2

Microscope –light ,electron and laser optic system Algae –and bacteria, fungi, and protozoa - morphology, ultra structure , sub cellular structure and cell envelope-slime, capsule, cell wall, cell inclusion. reproduction and life cycle pattern .

MICROSCOPE

INTRODUCTION TO LIGHT MICROSCOPY

- The light microscope is an instrument used by researchers in many different fields to magnify specimens to as much as a thousand times their original size. In its simplest form, it is composed of a clear lens that magnifies the sample and a light source to illuminate it.
- The basic principles of magnification, focus, and resolution are also introduced. Basic light microscope operation begins with bringing light to the sample and ensuring that the light source is of the correct intensity, directionality, and shape in order to produce the best quality image.
- Next, the sample must be magnified properly and brought into focus to view the region of interest.
- There are many practical applications for light microscopy including the viewing of stained or unstained cells and tissues, resolving small details of specimens, and even magnifying a region of interest during surgery to assist with complex procedures on the micron scale.

WHAT IS LIGHT MICROSCOPE?

- A light microscope is a biology laboratory instrument or tool, that uses visible light to detect and magnify very small objects, and enlarging them.
- They use lenses to focus light on the specimen, magnifying it thus producing an image. The specimen is normally placed close to the microscopic lens.

- Microscopic magnification varies greatly depending on the types and number of lenses that make up the microscope. Depending on the number of lenses, there are two types of microscopes.
- The functioning of the light microscope is based on its ability to focus a beam of light through a specimen, which is very small and transparent, to produce an image.
- The image is then passed through one or two lenses for magnification for viewing. The transparency of the specimen allows easy and quick penetration of light. Specimens can vary from bacterial to cells and other microbial particles.

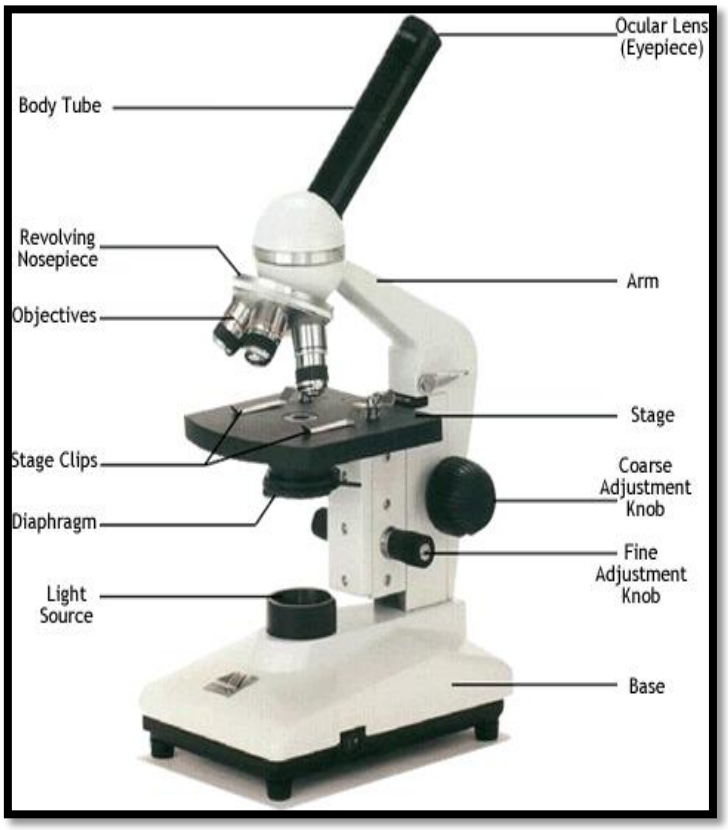
The light microscope is an important tool in the study of microorganisms, particularly for identification purposes.

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.

COMPOUND LIGHT MICROSCOPES TYPICALLY HAVE THE FOLLOWING COMPONENTS

- **Illuminator:** the light source in the base of the microscope;
- **Abbe Condensor:** a two lens system that collects and concentrates light from the illuminator and directs it to the iris diaphragm;
- **Iris diaphragm:** regulates the amount of light entering the lens system;
- **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;
- **Body tube:** houses the lens system that magnifies the specimens;
- **Upper end of body tube**—oculars/eye pieces: what you view through;
- **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:
- Magnification is simply the enlargement of the specimen. In a compound lens system, each lens sequentially enlarges or magnifies the specimen;
- The objective lens magnifies the specimen, producing a real image that is then magnified by the ocular lens resulting in the final image;
- The total magnification can be calculated by multiplying the objective lens value by the ocular lens value.



Always carry a microscope with one hand holding the arm and one hand under the base.

ELECTRON MICROSCOPE

Electron microscopy (EM) is a **technique for obtaining high resolution images of biological and non-biological specimens**. It is used in biomedical research to investigate the detailed structure of tissues, cells, organelles and macromolecular complexes.

DEFINITION

- An electron microscope is a microscope that uses a beam of accelerated electrons as a source of illumination.
- It is a special type of microscope having a high resolution of images, able to magnify objects in nanometres, which are formed by controlled use of electrons in vacuum captured on a phosphorescent screen.
- Ernst Ruska (1906-1988), a German engineer and academic professor, built the first Electron Microscope in 1931, and the same principles behind his prototype still govern modern EMs.

PRINCIPLE OF ELECTRON MICROSCOPY

- Electrons are such small particles that, like photons in light, they act as waves.

- **A beam of electrons passes through the specimen, then through a series of lenses that magnify the image**.
- The image results from a scattering of electrons by atoms in the specimen.
- There are several different types of electron microscopes, including the **transmission electron microscope (TEM), scanning electron microscope (SEM)**, and reflection electron microscope (REM.)

WORKING PRINCIPLE OF ELECTRON MICROSCOPE

Electron microscopes use signals arising from the interaction of an electron beam with the sample to obtain information about structure, morphology, and composition.

1. The electron gun generates electrons.
2. Two sets of condenser lenses focus the electron beam on the specimen and then into a thin tight beam.
3. To move electrons down the column, an accelerating voltage (mostly between 100 kV-1000 kV) is applied between tungsten filament and anode.
4. The specimen to be examined is made extremely thin, at least 200 times thinner than those used in the optical microscope. Ultra-thin sections of 20-100 nm are cut which is already placed on the specimen holder.
5. The electronic beam passes through the specimen and electrons are scattered depending upon the thickness or refractive index of different parts of the specimen.
6. The denser regions in the specimen scatter more electrons and therefore appear darker in the image since fewer electrons strike that area of the screen. In contrast, transparent regions are brighter.
7. The electron beam coming out of the specimen passes to the objective lens, which has high power and forms the intermediate magnified image.
8. The ocular lenses then produce the final further magnified image.

WHY WOULD YOU USE AN ELECTRON MICROSCOPE?

Electron microscopes are used to investigate the ultrastructure of a wide range of biological and inorganic specimens including microorganisms, cells, large molecules, biopsy samples, metals, and crystals. Industrially, electron microscopes are often used for quality control and failure analysis.

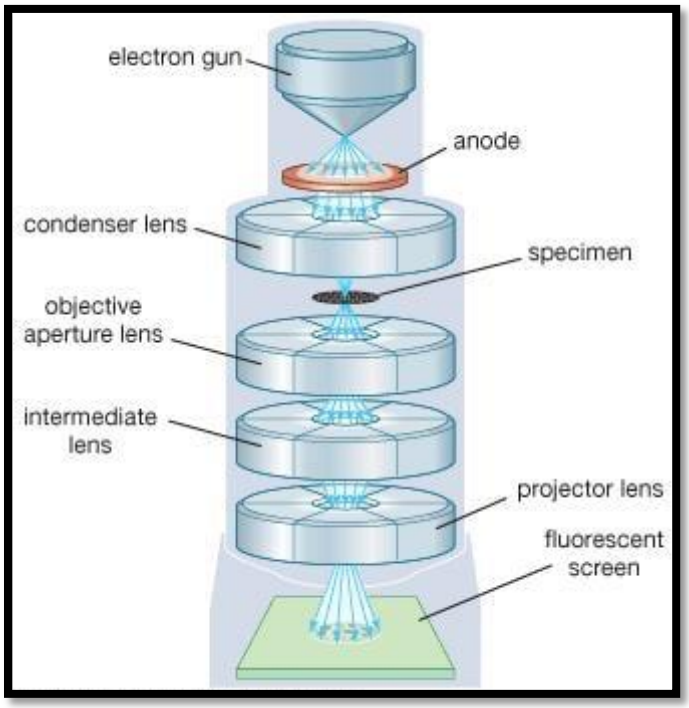
THERE ARE TWO MAIN TYPES OF ELECTRON MICROSCOPE

- The transmission EM (TEM) and the scanning EM (SEM). The transmission electron microscope is used to view thin specimens (tissue sections, molecules, etc) through which electrons can pass generating a projection image.
- The TEM is analogous in many ways to the conventional (compound) light microscope. TEM is used, among other things, to image the interior of cells (in thin sections), the structure of protein molecules (contrasted by metal shadowing), the organization of

molecules in viruses and cytoskeletal filaments (prepared by the negative staining technique), and the arrangement of protein molecules in cell membranes (by freeze-fracture).

There are two types of electron microscopes, with different operating styles:

1. THE TRANSMISSION ELECTRON MICROSCOPE (TEM)



- The transmission electron microscope is used to view thin specimens through which electrons can pass generating a projection image.
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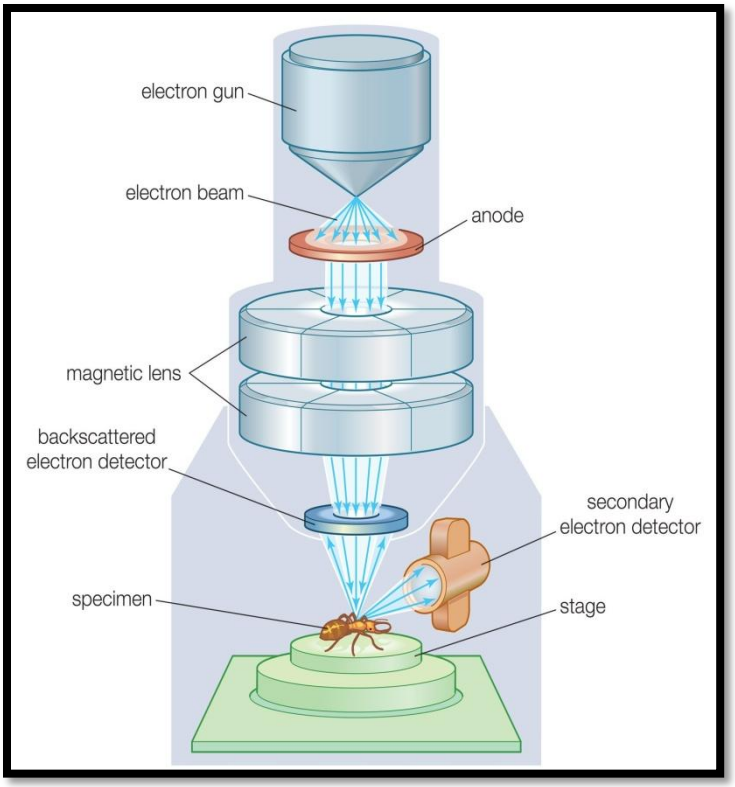
- TEM is used, among other things, to image the interior of cells (in thin sections), the structure of protein molecules (contrasted by metal shadowing), the organization of molecules in viruses and cytoskeletal filaments (prepared by the negative staining technique), and the arrangement of protein molecules in cell membranes (by freeze-fracture)

2. THE SCANNING ELECTRON MICROSCOPE (SEM)



- Conventional scanning electron microscopy depends on the emission of secondary electrons from the surface of a specimen.
- Because of its great depth of focus, a scanning electron microscope is the EM analog of a stereo light microscope.
- It provides detailed images of the surfaces of cells and whole organisms that are not possible by TEM. It can also be used for particle counting and size determination, and for process control.
- It is termed a scanning electron microscope because the image is formed by scanning a focused electron beam onto the surface of the specimen in a raster pattern.

PARTS OF ELECTRON MICROSCOPE

EM is in the form of a tall vacuum column which is vertically mounted. It has the following components:

1. **Electron gun**

- The electron gun is a heated tungsten filament, which generates electrons.

2. **Electromagnetic lenses**

- **Condenser lens** focuses the electron beam on the specimen. A second condenser lens forms the electrons into a thin tight beam.
- The electron beam coming out of the specimen passes down the second of magnetic coils called the **objective lens**, which has high power and forms the intermediate magnified image.

- The third set of magnetic lenses called **projector (ocular) lenses** produce the final further magnified image.
- Each of these lenses acts as an image magnifier all the while maintaining an incredible level of detail and resolution.

3. **Specimen Holder**

- The specimen holder is an extremely thin film of carbon or collodion held by a metal grid.

4. **Image viewing and Recording System.**

- The final image is projected on a fluorescent screen.
- Below the fluorescent screen is a camera for recording the image.

APPLICATION

Electron microscopes are used to investigate the ultrastructure of a wide range of biological and inorganic specimens including microorganisms, cells, large molecules, biopsy samples, metals, and crystals.

- Industrially, electron microscopes are often used for quality control and failure analysis.
- Modern electron microscopes produce electron micrographs using specialized digital cameras and frame grabbers to capture the images.
- Science of microbiology owes its development to the electron microscope. Study of microorganisms like bacteria, virus and other pathogens have made the treatment of diseases very effective.

ADVANTAGES

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

LIMITATIONS

- The live specimen cannot be observed.
- As the penetration power of the electron beam is very low, the object should be ultra-thin. For this, the specimen is dried and cut into ultra-thin sections before observation.
- As the EM works in a vacuum, the specimen should be completely dry.
- Expensive to build and maintain
- Requiring researcher training
- Image artifacts resulting from specimen preparation.
- This type of microscope is a large, cumbersome extremely sensitive to vibration and external magnetic fields.

HOW ARE ELECTRON MICROSCOPES USED TODAY?

In life sciences, electron microscopy can be **used to explore the molecular nature and mechanisms of disease**, view the 3D structure of biological tissues or cells, determine the structure of proteins and observe viruses in a biological context.

LASER OPTIC SYSTEM

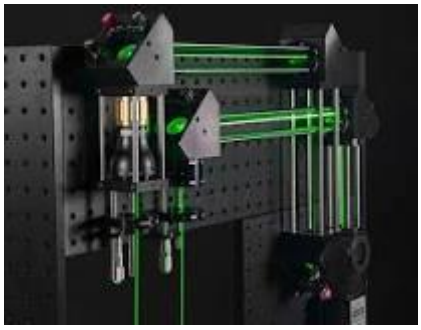
HISTORY

- Invented in 1958 by Charles towns N.P.1964 and Arthur Schawlow
- Based on Einsteins ideas of the particle wave duality of light.
- Originally called MASER (M=Microwave)

INTRODUCTION

- Light amplification by stimulated emission of radiation
- Laser, a device that stimulates atoms or molecules to emit light at particular wavelengths and amplifies that light, typically producing a very narrow beam of radiation. ... Laser is an acronym for "**light amplification by the stimulated emission of radiation**."

WHAT IS A LASER OPTICS?



- Laser Optics are used in a wide range of laser instrumentation or laser applications, including beam steering or material processing. Laser Optics use specific substrates, coatings, or a combination of the two to provide superior performance at specific laser wavelengths or over a range of wavelengths.

WHAT ARE LASER OPTICS MADE OF?

- laser windows (UV/VIS, VIS/NIR and IR windows made from MgF_2, CaF_2 or UV fuses silica, optical glass or fused silica, Ge, ZnSe, sapphire, silicon) laser mirrors (with dielectric coating, enhanced Al coatings, protective silver or gold coating, low Group Delay Dispersion (GDD) coatings)

HOW DOES LASER OPTICS WORK?

- An optical switch traps the low-energy laser pulse in the main amplifier for four passes through the laser glass slabs. Mirrors at both ends of the glass amplifier cause the photons

to travel back and forth through the glass, stimulating more electrons to drop to their lower energy states and emit photons.

- Laser optics essentially means optical elements and systems which are used with lasers – either as parts of lasers or for transmitting and manipulating laser beams or other forms of laser light.

OPTICS IN LASERS

A range of passive optical components is often used in lasers:

- Laser mirrors are often used for constructing laser resonators. Most of them are highly reflecting dielectric mirrors, while others have some partial transmisivity for use as output couplers. Dichroic mirrors are often used for injecting pump light into a laser resonator. For ultrafast lasers, one often requires dispersive mirrors.
- Lenses are not used much in laser resonators; focusing or defocusing is more often done with curved mirrors in order to minimize propagation losses and parasitic reflections.
- Prisms are more often used outside lasers, but sometimes also inside, particularly for dispersion compensation in ultrafast lasers.
- Wavelength tuning is often achieved by inserting some kind of optical filter in a laser resonator – for example, an etalon or a Lyot filter.
- Passive mode locking can be done by using a saturable absorber.
- Further, some kind of laser gain medium is required, which may for example be a laser crystal, a rare-earth doped fiber, a semiconductor gain chip (e.g. in an external-cavity diode laser or a vertical external-cavity surface-emitting laser) or a gas discharge tube.
- In some cases, one requires some kind of optical modulator – for example, an acousto-optic or electro-optic modulator for Q switching or mode locking.

OPTICS FOR LASER LIGHT

Outside a laser resonator, laser light often needs to be transported and manipulated, for which different kinds of optical components and systems can be used:

- Mirrors are used for redirecting laser light, also for precisely adjusting the beam path. For example, one often uses a pair of mirrors, each one changing the beam direction by approximately 90°. Fine alignment of the beam is possible with micrometers screws on the mirror holders.
- Lenses (including cylindrical lenses) are often used for collimating a laser beam, or for modifying its beam radius, or for tight beam focusing. Sometimes, such things are done with complete assemblies like beam collimators, beam expanders and focusing objectives, which may contain multiple lenses.

- Anamorphic prism pairs may be used for converting elliptical beam profiles into circular ones.
- Mode cleaners can be used for improving the beam quality.
- Polarizing or non-polarizing beam splitters can be used for obtaining multiple beams or for guaranteeing linear polarization states.
- Waveplates can be used for manipulating the polarization state – for example, for rotating the polarization direction or converting linearly polarized light into circularly polarized light.
- Optical filters may be used to remove unwanted spectral components – for example, residual laser light after a frequency doubler.
- Neutral density filters and other optical attenuators can be used to reduce the optical power. There are also so-called noise eaters which automatically adjust the attenuation such as to obtain a constant output power.
- Faraday isolators are used for protecting a laser source against back-reflected light.
- Various kinds of optical modulators may be inserted, for example intensity or phase modulators or optical switches.
- In some cases, diffractive optics are used, for example for splitting a beam into a large number of beams with a single optical component.
- Unwanted beams may be sent into beam dumps for safely converting the optical energy into heat.
- There are scanning lenses for laser scanners, sending laser beams in variable directions.

MICROMETRY

Micrometry refers to the measurement of dimensions of the desired microorganisms under a microscope which uses two micro-scales known as 'micrometers'. At first, the diameter of the microscopic field must be established with the help these micrometers namely ocular micrometer and stage micrometer.

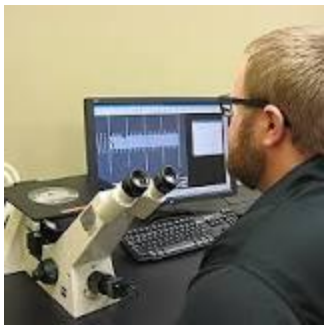
WHO INVENTED THE MICROMETER?

William Gascoigne

WHAT IS THE PRINCIPLE OF MICROMETRY?

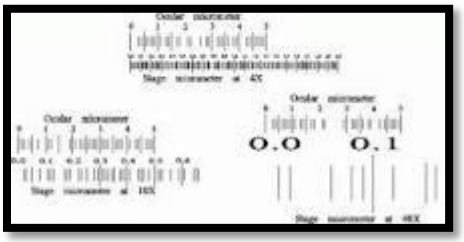
A micrometer works on the principle of a screw and a nut. It allows you an axial rotation of the barrel-like structure, also known as Thimble, which is used to measure the distance of the object

WHAT IS CALIBRATION IN MICROMETRY?



Microscope calibration includes a comparison of the grid or scale on the eyepiece reticle with the scale markings of a known dimension on a stage micrometer. ... In order to use these units to measure an object with the microscope, reticle calibration is required to calibrate the scale.

WHAT IS OCULAR AND STAGE MICROMETER?



To measure an object seen in a microscope, an ocular micrometer serves as a scale or rule. ... To use the ocular micrometer, calibrate it against a fixed and known ruler, the stage micrometer. Stage micrometers also come in varying lengths, but most are 2 mm long and subdivided into 0.01 mm (10 micrometer) lengths

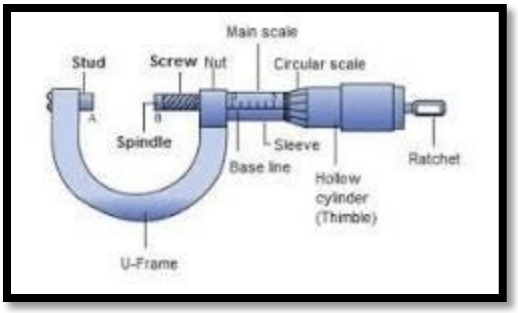
WHAT ARE THREE TYPES OF MICROMETERS?

There are many types of micrometer available, but the most common types include:-Outside Micrometer; Depth Micrometer; Inside Micrometer; Bore Micrometer; Outside micrometer.

WHY IS MICROMETRY IMPORTANT IN RESEARCH?

Micrometry is the science in which we have some measurement of the dimensions of an object being observed under the microscope. The method employs some special types of measuring devices which are so oriented that these can well be attached to or put into the microscope and observed.

WHAT IS LEAST COUNT MICROMETER?



Micrometer Screw gauge: Micrometer screw gauge is an instrument used to measure the diameter of thin wires, the thickness of small sheets of glass, plastic, etc. It can measure up-to 1/10 of mm (or **0.01 mm= 0.001 cm**) which is usually called the least count of Micrometer.

WHAT'S A MICROMETER USED FOR?

A micrometer is a measuring instrument that can make extraordinarily precise measurements. Most micrometers are designed to measure within one one-thousandth of an inch! That's a close fit. Exact measurements like this are necessary when even the smallest of space between objects can cause problems or difficulties.

HOW DO YOU CALCULATE MICROMETER?

You just have to add the first part and second part of the measurement to obtain the micrometer reading: 5.5+0.28=5.78 5.5 + 0.28 = 5.78 mm.

WHAT ARE APPLICATIONS OF OUTSIDE MICROMETER?

Outside Micrometers are used for measuring the thickness or outside diameter of small parts. They are industry standard measuring tools because of their high accuracy/resolution and ease of use.

ALGAE

THE MORPHOLOGY OF ALGAE

- In the not too distant future, advances in DNA identification could potentially change the way that ambiguous organisms like algae are classified.
- In the meantime, phycologists will continue to rely on a naming and classification system of morphology introduced by Carl Linnaeus in the 1700s.
- Like other members of the kingdom Protista, algae are eukaryotic organisms with a nuclear envelope, cell walls and organelles.

MAIN CHARACTERISTICS OF ALGAE

- Algae are protists, an incredibly large group of organisms with markedly different features. The form and structure of algae sets them apart from plants. Although algae and plants both contain chlorophyll and photosynthesize, algae don't have an actual root system, stem or leaves. Algae cells are typically simpler than plant cells and have fewer organelles in their cell cytoplasm.
- There are few places on Earth where algae cannot be found. Algae thrive in places where few plants would dare to go. Habitats include everything from the deepest ocean to snowy mountain caps to hot springs and salt marshes.
- Most species of algae are single-celled micro-organisms living in aquatic environments. Algae are primary producers on the bottom of the food chain that feed consumers. Algae are often distinguishable by their color.

GOLDEN BROWN ALGAE (CHRYSOPHYTES)

- Golden algae (Chrysophytes) are common microscopic organisms that provide food for zooplankton in fresh water.
- Most are functionally photosynthetic, but under the right conditions, golden algae feed on bacteria. Structurally, golden algae are mostly unicellular and free-swimming, but some species exist as colonial algae and stringy filaments.
- Chrysophytes like diatoms can be seen in fossil records dating back to the Cretaceous age.

COMMON GREEN ALGAE

- More than 7,000 species of green algae have been identified, according to the UC Museum of Paleontology.
- Freshwater green algae like Spirogyra in the Charophyta phylum are more closely related to plants than marine green algae (Chlorophyta).
- Green algae resembles a plant because it contains chlorophyll and uses sun energy to drive photosynthesis.
- The structure of green algae can be single- or multiple-celled.

RED ALGAE (RHODOPHYTA)

- The typical red algae (Rhodophyta) is a rose-colored multicellular organism found in marine environments around the world.
- Accessory pigments called *phycobiliproteins* are responsible for the distinctive red coloring. Like green algae, red algae traces back to ancestral cyanobacteria.
- Certain types of red algae are edible and used to make products like agar and food additives.

BROWN ALGAE (PHAEOPHYTA)

- Brown algae (Phaeophyta) are multicellular organisms that derive their color from the brownish pigment *fucoxanthin* in chloroplasts along with chlorophyll.
- According to the Seaweeds of Alaska website for phycologists, brown algae are bigger and more morphologically complex than any other type of marine algae.
- Brown algae make their food through photosynthesis and store polymers of glucose in a vacuole within the cell cytoplasm.
- Familiar examples of brown algae are seaweed and kelp.

FIRE ALGAE (PYRROPHYTA)

- Phytoplankton are microalgae divided into two subgroups: diatoms and dinoflagellates. Phytoplankton play an important role in the food chain and ecosystem by converting nitrates, sulfur and phosphates into carbon-based nutrients.
- Runoff from farm fields and other pollutants can result in phytoplankton overgrowth and the formation of highly toxic harmful algal blooms (HABs).
- Deadly HABs, referred to as "red tides," form large, putrid-smelling masses over bodies of water. Bioluminescent types of dinoflagellates are called fire algae because they chemically emit light and glow like flames. At night the bioluminescent HAB appears on fire.

YELLOWISH GREEN ALGAE (XANTHOPHYTA)

- Xanthophyta are yellow-green algae that live in fresh water. They may be unicellular in morphology or colonial algae, bunched together.
- Color is derived from green, yellow and orange pigments involved in photosynthesis. Flagella make this type of algae motile in water.

WHAT IS THE STRUCTURE OF AN ALGAE?

ALGAL CELL STRUCTURE

Algae are eukaryotic cells, or cells that **contain a nucleus**, which makes them slightly more complex than bacteria. They also contain chloroplasts, which are structures that generate energy for the cell through photosynthesis. Other structures that algae may have vary greatly

WHAT CAUSES SLIME ALGAE?

Red Slime Algae is actually a bacteria. Cyanobacteria, to be specific. Elevated waste levels including both phosphate and nitrate are the leading reason this slimy red film grows in your tank. Lack of proper water circulation and old light bulbs also cause excessive growth of Cyanobacteria.

ALGAE CAPSULES

Algae Capsules is a natural food supplement containing high valuable seaweed extracts of Ascophyllum nodosum and is free of artificial chemical product. Capsules are made from cellulose and ideal for vegetarian and vegan people. Gluten and lactose free.

WHAT IS THE OUTER COVERING OF ALGAE?

lorica

It is the non-living component of cell. Some golden algae, ciliates and choanoflagellates produces a shell-like protective outer covering called lorica. Some dinoflagellates have a theca of cellulose plates, and coccolithophorids have coccoliths.

WHAT IS THE MORPHOLOGY OF ALGAE?

Algae exhibit a very wide range of morphological diversity. The simplest forms are **unicellular, microscopic, motile or non-motile eukaryotic cells**. They may be spherical (Protococcus, Chlorella), or pyriform (Chlamydomonas).

What are 5 characteristics of algae?

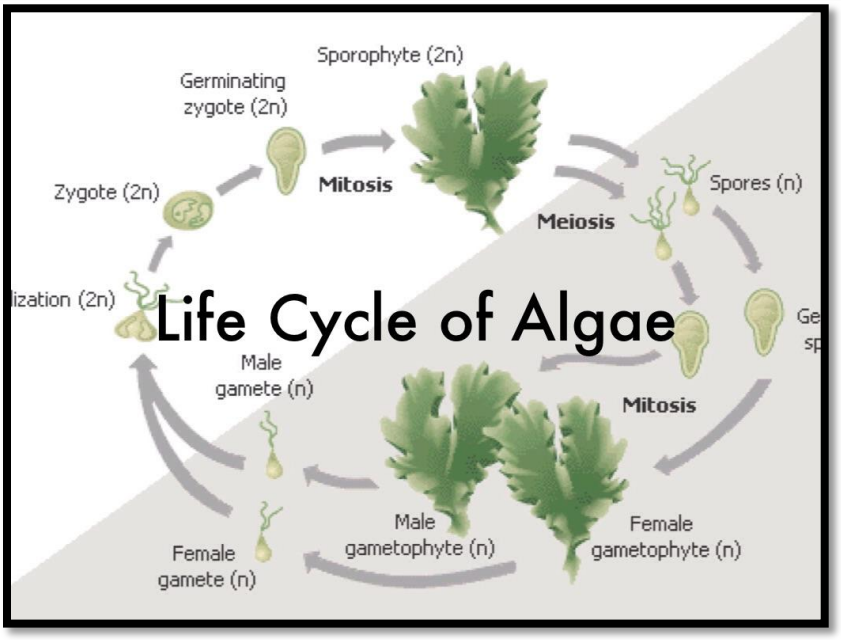
CHARACTERISTICS OF ALGAE

- **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.
- Algaes are found where there is adequate moisture.
- Reproduction in **algae** occurs in both asexual and sexual forms.

WHAT ARE THE MAIN FEATURES OF ALGAE?

- they are primarily aquatic.
- algae are photosynthetic organism.
- they are thallophytic means without true roots,stems,and leaves.
- they are cryptogamic means they do not produce seeds, flowers.
- they possess a photosynthetic apparatus called pyrenoid.
- it exhibit both sexual and asexual reproduction.

LIFE CYCLE OF ALGAE



Algae are one of the most diverse species of plant life. They are photosynthetic, like most other plants, but lack most of the structures of terrestrial plant life, such as stalks, leaves and rhizomes. All algae goes through a haploid life cycle of development, starting with a diploidzygote, or spore, and ending up with a fully mature alga plant.

There are three main classifications of algae: Rhodophyta, Cholorphyta and Heterokontophyta.

DEVELOPMENT

The first step in the development process takes place when the diploid, or immature spore cell, goes through a cellular division process known as meiosis. Before this process, the diploid is actually known as a diploidzygote. Afterward, it's called a haploid spore.

During meiosis, the single diploidzygote transforms from a single cell into four distinct and separate cells or spores. These haploid cells are now sexually mature and ready to mate. The male and female haploids fuse together to form gametes.

After fusion, the gametes form new diploid cells and the process begins again.

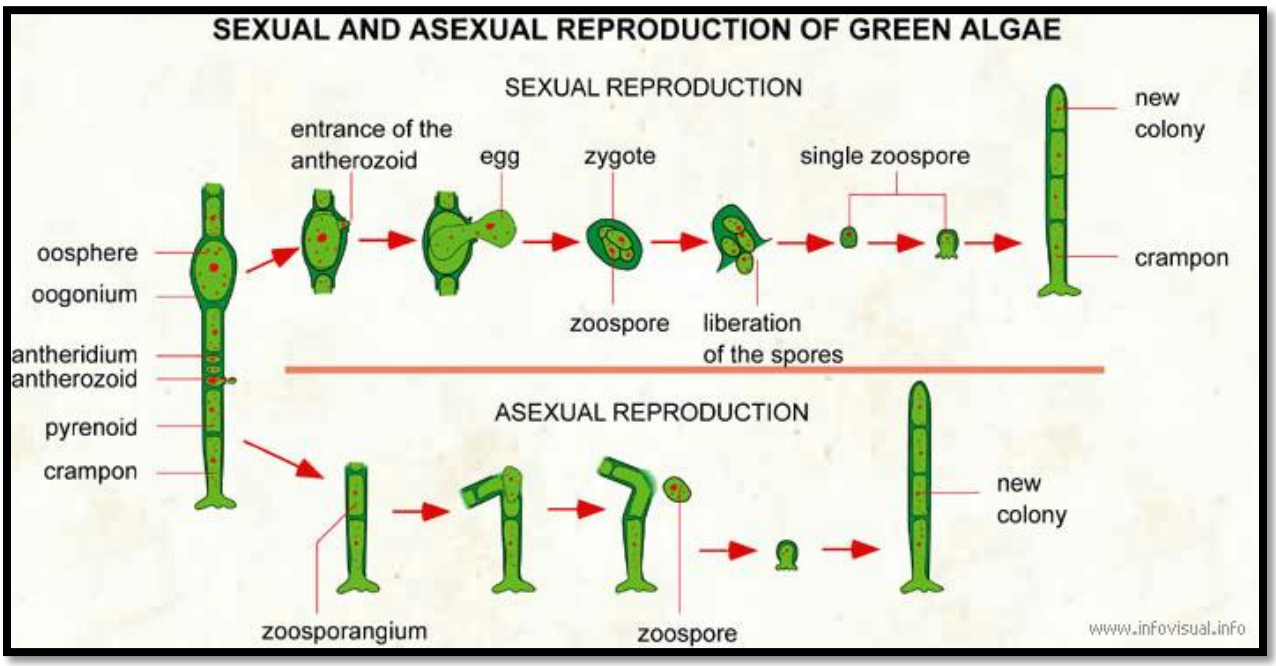
Life spans differ for each species of algae, with an average life expectancy ranging from a few days to a year or two.

REPRODUCTION

Algae can reproduce in one of two ways, either asexually by mitosis or sexually, with the fusion of the gametes. Asexual reproduction can happen much more quickly, but diversity is limited. Sexual reproduction allows for greater diversity but is considerably slower.

HOW DO ALGAE REPRODUCE?

Algae are a large group of simple plant-like organisms that reproduce in a surprisingly varied number of ways, both sexually and asexually. Some species alternate between reproduction methods in succeeding generations. Algae may exist as single-celled organisms called plankton, may form colonial organisms such as seaweed, or may join with fungi to form lichens. Different species can dwell in fresh water, seawater or moist rock.



ASEXUAL CELL DIVISION

In asexual reproduction, the genetic material of the parent cell doesn't combine with that from another cell. The simplest method of reproduction that algae employ is asexual binary fission, in which a cell splits into two, either at the equator or along its length. In some species, a rapid series of divisions results in small groupings. Asexual reproduction can also take place when algae fragment into pieces, or when special cells bud off from a colony to form new individuals.

ASEXUAL SPORES

- Many species of algae can form special cells called spores. In asexual reproduction, the spores can produce new individuals without the need for another parent, as would be the case in sexual reproduction.
- The asexual spores normally contain two sets of chromosomes, which are structures that house the individual's genetic material.
- One type of spore has flagella -- little whip-like tails -- that enable movement.
- Another type develops inside the parent cell without flagella, which they can grow after separating from the parent. A third type of algae does not develop flagella and therefore lacks self-propulsion.

SEXUAL REPRODUCTION

- In sexual reproduction, two individuals each contribute one set of chromosomes that unite to create offspring with two sets of chromosomes, having traits from both parents.
- The simplest algal sexual method, conjugation, occurs when two individuals fuse, share genetic material and then separate.
- The fusion in some species takes place via special tubes.
- In most multicellular species of algae, individuals produce special sex cells, called gametes, that contain only one set of chromosomes.
- The gametes from two individuals fuse sexually and can develop directly into offspring, or they can form cells that subsequently produce spores.

COMBINATION SEX

- Some species of algae reproduce through a mechanism having both sexual and asexual stages. In this method, a mature cell has just one set of chromosomes rather than the customary two. Through cell division, one parent cell can create four spore cells, each having one set of chromosomes and ready for sexual fusion with other spores. Some other species reproduce in a two-cycle pattern called "alternation of generations.
- " In the first cycle, cells form gametes asexually. These fuse in the next cycle to form cells with two sets of chromosomes. These develop into mature cells that produce spores with a single set of chromosomes, bringing the process full circle.

BACTERIA

DEFINITION OF BACTERIA

- Bacteria are microscopic unicellular organism they are true living organism that belongs to the kingdom prokaryotes.
- (Singular: bacterium) are a large group of unicellular microorganisms.
- They are extremely tiny thus they cannot be seen individually unless viewed through microscope.
- When cultured on agar, the bacteria grow as colonies that contain many individual cells. These colonies appear as spots of varying size, shape and colour, depending on the microorganism.

MORPHOLOGY OF BACTERIA:

- Bacteria are very small unicellular microorganisms ubiquitous in nature. They are micrometres ($1\mu m = 10^{-6}$ m) in size.
- They have cell walls composed of peptidoglycan and reproduce by binary fission. Bacteria vary in their morphological features.

THE MOST COMMON MORPHOLOGIES ARE:

Coccus (Pleural – Cocci):

- Spherical bacteria; may occur in pairs (diplococci), in groups of four (tetracocci), in grape-like clusters (Staphylococci), in chains (Streptococci) or in cubical arrangements of eight or more (sarcinae).
- For example – Staphylococcus aureus, Streptococcus pyogenes.

Bacillus (Pleural – Bacilli):

- Rod-shaped bacteria; generally occur singly, but may occasionally be found in pairs (diplo-bacilli) or chains (streptobacilli).
- For example – Bacillus cereus, Clostridium tetani.

Spirillum (Pleural – Spirilla):

- Spiral-shaped bacteria.
- For example – Spirillum, Vibrio, Spirochete species.

Some Bacteria have Other Shapes Such as:

- Coccobacilli – Elongated spherical or ovoid form.
- Filamentous – Bacilli that occur in long chains or threads.
- Fusiform – Bacilli with tapered ends.

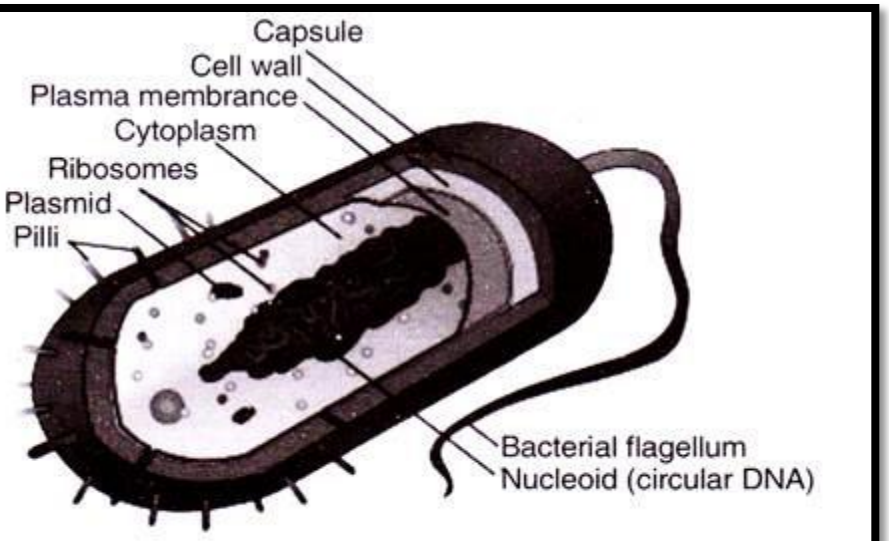


Fig. 11.1: Structure and contents of a typical gram positive bacteria cell

(i) Most numerous organisms on earth.
(ii) Earliest life forms (fossils date 2.5 billion years old).
(iii) Microscopic prokaryotes (no nucleus non membrane-bound organelles).
(iv) Contain ribosomes.
(v) Infoldings of the cell membrane carry on photosynthesis and respiration.
(vi) Surrounded by protective cell wall containing peptidoglycan (protein- carbohydrate).
(vii) Many are surrounded by a sticky, protective coating of sugars called the capsule or glycocalyx (can attach to other bacteria or host).
(viii) Have only one circular chromosome.
(ix) Have small rings of DNA called plasmids.
(x) May have short, hair like projections called pili on cell wall to attach to host or another bacteria when transferring genetic material.
(xi) Most are unicellular.
(xii) Found in most habitats. (xiii) Most bacteria grow best at a pH of 6.5 to 7.0.
(xiv) Main decomposers of dead organisms so recycle nutrients.
(xv) Some bacteria breakdown chemical and oil spills.
(xvi) Some cause disease.
(xvii) Move by flagella
(xviii) Some can form protective endospores around the DNA when conditions become unfavorable; may stay inactive several years and then re-activate when conditions favorable.
(xix) Classified by their structure, motility (ability to move), molecular composition, and reaction to stains (Gram stain).
(xx) Grouped into 2 kingdoms – Eubacteria (true bacteria) and Archaebacteria (ancient bacteria).
(xxi) Once grouped together in the kingdom Monera.

(xxii) Classified by their structure, motility (ability to move), molecular composition, and reaction to stains (Gram stain).

ULTRASTRUCTURE OF A BACTERIAL CELL

The bacterial cell reveals three layers (i) Capsule/Glycocalyx (ii) Cell wall and (iii) Cytoplasm (Figure 1.9)

CAPSULE/GLYCOCALYX

Some bacteria are surrounded by a gelatinous substance which is composed of polysaccharides or polypeptide or both. A thick layer of **glycocalyx** bound tightly to the cell wall is called **capsule**.

It protects cell from desiccation and antibiotics. The sticky nature helps them to attach to substrates like plant root surfaces, Human teeth and tissues. It helps to retain the nutrients in bacterial cell.

CELL WALL

- The bacterial cell wall is granular and is rigid.
- It provide protection and gives shape to the cell.
- The chemical composition of cell wall is rather complex and is made up of Peptidoglycan or mucopeptide (N-acetyl glucosamine, N-acetyl muramic acid and peptide chain of 4 or 5 aminoacids).
- One of the most abundant polypeptide called porin is present and it helps in the diffusion of solutes.

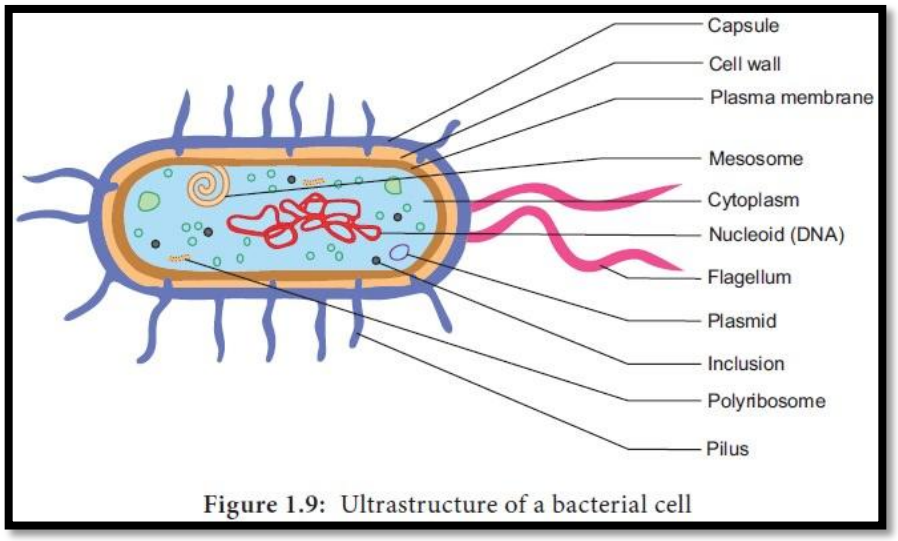


Figure 1.9: Ultrastructure of a bacterial cell

PLASMA MEMBRANE

The plasma membrane is made up of lipoprotein. It controls the entry and exit of small molecules and ions. The enzymes involved in the oxidation of metabolites (i.e., the respiratory chain) as well as the photosystems used in photosynthesis are present in the plasma membrane.

CYTOPLASM

Cytoplasm is thick and semitransparent. It contains ribosomes and other cell inclusions. Cytoplasmic inclusions like glycogen, poly-β-hydroxybutyrate granules, sulphur granules and gas vesicles are present.

BACTERIAL CHROMOSOME

- The bacterial chromosome is a single circular DNA molecule, tightly coiled and is not enclosed in a membrane as in Eukaryotes.
- This genetic material is called **Nucleoid or Genophore.**
- It is amazing to note that the DNA of *E.coli* which measures about 1mm long when uncoiled, contains all the genetic information of the organism.
- The DNA is not bound to **histone** proteins.
- The single chromosome or the DNA molecule is circular and at one point it is attached to the plasma membrane and it is believed that this attachment may help in the separation of two chromosomes after DNA replication.

PLASMID

- Plasmids are extra chromosomal double stranded, circular, self-replicating, autonomous elements. They contain genes for fertility, antibiotic resistant and heavy metals. It also help in the production of bacteriocins and toxins which are not found in bacterial chromosome.
- The size of a plasmid varies from 1 to 500 kb usually plasmids contribute to about 0.5 to 5.0% of the total DNA of bacteria. The number of plasmids per cell varies.
- Plasmids are classified into different types based on the function. Some of them are F (Fertility) factor, R (Resistance) plasmids, Col (Colicin) plasmids, Ri (Root inducing) plasmids and Ti (Tumour inducing) plasmids.

MESOSOMES

These are localized infoldings of plasma membrane produced into the cell in the form of vesicles, tubules and lamellae. They are clumped and folded together to maximize their surface area and helps in respiration and in binary fission.

POLYSOMES / POLYRIBOSOMES

- The ribosomes are the site of protein synthesis.
- The number of ribosome per cell varies from 10,000 to 15,000.
- The ribosomes are 70S type and consists of two subunits (50S and 30S).

- The ribosomes are held together by mRNA and form polyribosomes or polysomes.

FLAGELLA

- Certain motile bacteria have numerous thin hair like processes of variable length emerge from the cell wall called flagella. It is 20–30 µm in diameter and 15 µm in length.
- The flagella of Eukaryotic cells contain 9+2 microtubles but each flagellum in bacteria is made up of a single fibril.
- Flagella are used for locomotion.
- Based on the number and position of flagella there are different types of bacteria (Figure 1.8)

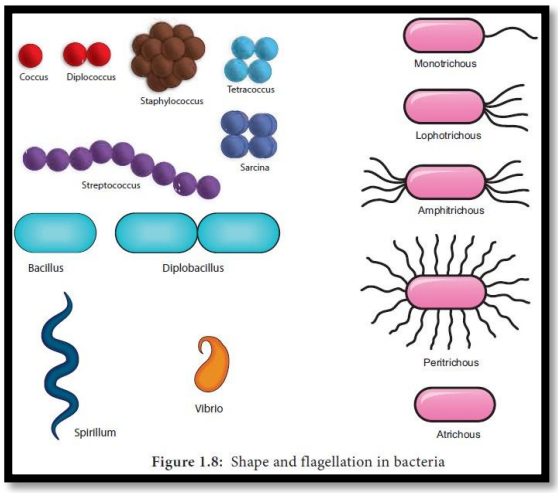


Figure 1.8: Shape and flagellation in bacteria

FIMBRIAE OR PILI

Pili or fimbriae are hair like appendages found on surface of cell wall of gram-negative bacteria (Example: *Enterobacterium*). The pili are 0.2 to 20 µm long with a diameter of about 0.025µm. In addition to normal pili there are special type of pili which help in conjugation called sex pili are also found.

BACTERIA REPRODUCTION

Just like any other organism, bacteria also reproduce to continue their species. Since they are unicellular and do not have a well-organised cell, bacteria have been grouped under prokaryotes. However, they do show both sexual and asexual means of reproduction. In this topic, we will have a brief overview of all types of means of reproduction in bacteria.

In asexual reproduction in bacteria, 5 methods are observed. These are:

1. Binary fission
2. Reproduction through conidia
3. Budding

4. Reproduction through cyst formation
5. Reproduction through endospore formation

However, in asexual reproduction, genetic recombination is not observed and that is why sexual reproduction has high significance in the continuation of a bacterial species. This is because, in sexual reproduction, genetic material is exchanged between two cells and which facilitates genetic recombination and creates a genetic drift in the species of a bacteria. There are 3 ways bacteria reproduce sexually, these are:

1. Transformation
2. Transduction
3. Conjugation

HOW DO BACTERIA REPRODUCE?

As we have already discussed, bacteria reproduce through both asexual and sexual means. IN this section we will learn about these different modes of reproduction in bacteria.

ASEXUAL REPRODUCTION IN BACTERIA

Binary Fission:

- In binary fission, a single bacterial cell divides into two daughter cells. At first, the bacterial cell reaches critical mass in its form and cell components.
- The circular double-stranded DNA of the bacteria undergoes replication and new complementary strands are formed.
- These two strands of DNA are then moved to the different poles of the cell and a transverse septum then takes place and develops in the middle region of the cell which separates the two new daughter cells and thus binary fission I completed.
- It is a rapid process and takes minutes to complete.

Conidia Formation:

- The formation of conidia takes place in filamentous bacteria such as Streptomyces through the formation of a transverse septum at the apex of the filament.
- The part bearing the conidia is called the conidiophore and after it is detached from the mother cell, in a suitable substratum it germinates giving rise to new mycelium.
- This type of asexual reproduction is also called fragmentation.

Budding:

- In this method of reproduction, the bacterial cell develops a small swelling at one side which continuously increases in size.
- At the same time, the nucleus also undergoes division where one part with some cytoplasm enters the swelling and the other part remains with the mother cell.
- The outgrowth is called the bud and it eventually gets separated from the mother cell by a partition wall.
- This method of reproduction also comes under vegetative reproduction in bacteria.
- Example: Rhodomicrobium vannielii

Cysts:

- Cysts are formed by the deposition of additional layers around the mother cell and are the resting structure during unfavourable conditions.
- When conditions are favourable again, the mother cell behaves like its normal self again. Example: Azotobacter.

Reproduction through endospore formation:

- Endospores in a bacterial cell are formed during stressful conditions such as desiccation and starvation.
- They contain a central protoplast, and a core consisting of DNA, ribosomes, enzymes and the t-RNA, everything necessary for the formation of a new cell.
- Only one endospore is formed in one bacterial cell and on germination, it gives rise to a new bacterial cell.

SEXUAL REPRODUCTION IN BACTERIA

Transformation:

In transformation, a bacterium takes up DNA from its environment and often DNA that's been shed by another bacteria. The phenomenon was first discovered by Griffith in 1928 and the mechanism was worked out by Avery in 1944. In this process, the DNA of a capsulated bacteria is transferred into a non-capsulated bacteria. If the DNA is circular it is called a plasmid. The plasmid can be copied in the receiving cell and passed on to its descendants.

Transduction:

In this type of sexual reproduction of bacteria, foreign genes are transferred into a bacterial cell with the help of a virus. These viruses are called bacteriophage and they are not virulent. The virus acts as a carrier vehicle and passes over genes from one host to another. Transducing bacteriophages may carry the same genes in which the reproduction method would be known as restricted transduction. They can also carry different genes at different times in which the reproduction process would be known as generalised transduction.

Conjugation:

This process was first discovered in Escherichia coli by Tatum and Lederberg in 1946. They found that two different types of nutritional mutants grown together on minimal medium produced an occasional wild type.

Bacteria that show conjugation are dimorphic, meaning that they have two types of cells, one male (F+) or donor cell and a female (F-) or recipient cell.

The male or donor cell possesses 1 to 4 sex pili on the surface and fertility factor (transfer factor, sex factor) in its plasmid. It contains genes for producing sex pili and other characters needed for gene transfer. Sex pili are 1to 4 narrow protoplasmic outgrowths. The sex pili and fertility factor are absent from the female or recipient cells.

If these two types of cells happen to come nearer, a pilus of a male cell establishes a protoplasmic bridge or conjugation tube with the female cell. It takes 6-8 minutes for the process to complete.

These were the three types of sexual reproduction in bacteria and it introduces genetic variation in a bacterial species which is important for the survival of any species and allows groups to adapt to environmental changes.

LIFE CYCLE OF BACTERIA

- The bacteria life cycle consists of the lag phase, the log or exponential phase, the stationary phase and the death phase. Factors that influence bacterial growth bear heavily on this cycle.

Lag Phase

- Bacteria do not grow during the lag phase. However, they do adjust to their environment and metabolize, that is, produce vitamins and amino acids needed for division. They begin making copies of their DNA, and if the environment supplies plenty of nutrients, the lag phase may be very short. Then the bacteria will proceed to the next phase of their life.

Log or Exponential Phase

- During the log or exponential phase, bacteria multiply rapidly, even exponentially. The time it takes for a culture to double is called "generation time," and under the best conditions, the fastest bacteria can double in about 15 minutes. Other bacteria take days.
- Within a bacterium, the DNA copy drifts to the opposite side of the membrane. The bacterium then pulls apart, creating two identical "daughter cells," which begin dividing anew. This process is called binary fission.

Stationary Phase

- During the stationary phase, bacteria growth dwindles. Due to accumulating waste and a lack of space, bacteria cannot maintain the clip of the log or exponential phase. If the bacteria moves to another culture, however, rapid growth may resume.

Death Phase

- During the death phase, bacteria lose all ability to reproduce, which becomes their death knell. Like the log or exponential phase, bacterial death may occur as rapidly as their growth.

FUNGI

INTRODUCTION

- Fungi are **heterotrophic** organisms which means they require organic compound for nutrition or growth.
- Fungi are **spore-bearing** eukaryotes.
- Fungi may be unicellular or multicellular.
- Fungi includes **moulds** and **yeast.**
    - **Molds**– filamentous, multicellular.
    - **Yeast**– unicellular.
- Study of fungi is generally known as **mycology.**

DISTRIBUTION

- The fungus occurs in all possible habitats i.e. aquatic, terrestrial (which grow in soil, on dead and decaying material).
- Some grow on plants and animals.
- Fungi also present in the air.

- In fungus chlorophyll are absent, so they depend on other for food. That is why fungi may be saprophytes, parasite or symbionts.

MORPHOLOGY

- Yeasts cells are generally larger than most of the bacteria.
- Size of yeast ranging from 1 to 5 micrometers in width and from 5 to 30 micrometers in length.
- Flagella or other organelles of locomotion are absent in yeast.
- Cell wall constituents of fungi are mainly chitin and glucans.
- Multicellular fungi are composed of networks of long filamentous branched structure called hyphae.
- The hyphae often aggregate in a thread like dense network known as mycelium.
- The hyphae may be:
    - Without crosswalls as in the case of lower fungi or, Divided into compartment by formation of septa in the higher fungi.
- Hyphae occurs in three forms:
    - Coenocytic or nonseptate, such hyphae have no septa.
    - Septate with uninucleate cells.
    - Septate with multinucleate cells.
- The mycelium forms tissue like aggregates called the **plectenchyma,** in certain stages, often during transition to the sexual or asexual reproduction phase.

REPRODUCTION

In fungi reproduction maybe **asexual** or **sexual**.

ASEXUAL REPRODUCTION

- Asexual reproduction also known as somatic or vegetative reproduction.
- It does not involve the sex cells or sex organs and the union of nuclei.
- Asexual reproduction maybe occurs by:
    - Fission of somatic cells.
    - Budding of somatic cells or spores.
    - Fragmentation or disjoining of the hyphal cells.
    - Spore formation.

There are many kinds of asexual spores:

1. Sporangiospores.
2. Conidiospores or conidia(conidium)**.**
3. oidia(oidium), arthrospores**.**
4. Chlamydospores**.**

5. Blastospores**.**

Sporangiospores

- Single-celled spores.
- Formed at the end of hyphae within sacs sporangia or sporangium.
- Aplanospores: Non-motile sporangiospores.
- Zoospores- motile.
- Example- Mucor, Rhizopus.

Conidiospores or conidia(conidium)

- Microconidia is small and single-celled conidia.
- Macroconidia- large, multicellular conidia.

They are formed at tip or side of the hyphae.

- Example– penicillium, aspergillus.
- oidia(oidium), arthrospores.These spores are single-celled formed by fragmentation of hyphae cells.Example– In the the milk mould *Endomyces lactis.*

Chlamydospores

- These single-celled sports are surrounded by thick walls are highly resistant in adverse condition.
- These are formed by vegetative hypha cells.

Blastospores

- Through boarding spores are formed.

SEXUAL REPRODUCTION

- It is carried out by fusion of genetic materials of two parent's cells.
- Sexual reproduction can be divided into three phases:
    - Plasmogamy- Joining of two cells and fusion of their protoplast.
    - Karyogamy- This involved fusion of two haploid nuclei.
    - Meiosis- Reduction of chromosomes to the haploid number.
- Gametangia- The sex organelles of fungi (if present).
- Antheridium- Male gametangia.
- Oogonium- Female gametangia.

There are various types of sexual spores

- **Ascospores-** Single-celled produced in sac known as **Ascus.**
- **Basidiospores-** These spores are single celled and borne on a **club- shaped** structure known as basidium or basidia.
- **Zygospores-** are big in size, thick-walled spores formed by fusion of 2 sexually compatible hyphae or gametangia.

- **Oospores** formed within **Oogonium,** produced by fertilization of oospheres by male gametes.

Fruiting bodies

- ❖ Fruiting bodies are highly organised protective structure in which sexual and sexual spores may be surrounded.
- ❖ **Acervulus** and **pycnidium-** asexual fruiting bodies.
- ❖ **Perithecium** and **apothecium-** sexual fruiting bodies.

CLASSIFICATION

Fungi are classified in four major divisions:

1. **Chytridiomycota**
2. **Zygomycota**
3. **Ascomycota**
4. **Basidomycota**

5.Chytridiomycota

- It is commonly known as **chytrids**.
- **Habitat-** Aquatic or terrestrial.
- **Ploidy-** Diploid.
- **Motile stage-** Present.
- **Asexual spores-** Holocarpic.
- **Pathogenic relationship-** Obligate parasite.

ZYGOMYCOTA

- These are known as **zycomycetes.**
- **Habitat**– Terrestrial.
- **Ploidy**– Monoploid
- **Motile stage**– Absent.
- **Sexual spore**– Zygospores.
- **Asexual spore**– Sporangiospores/Chlamydospores.
- **Parasitic relationship**– Facultative parasite.
- **Example**– Black bread mould ***Rhizopus stolonifer***.

ASCOMYCOTA

- Commonly known as **Ascomycetes** or **sac fungi.**
- **Habitat**– Terrestrial.
- **Ploidy**– Monoploid.

- **Motile stage**– Absent.
- **Sexual spore**– Ascospores.
- **Asexual spore**– Conidia.
- **Pathogenic relationship** is facultative or obligate.

BASIDOMYCOTA

- It is commonly Known as basidiomycetes or **club – fungi.**
- **Habitat**– Terrestrial.
- **Ploidy**– Monoploid, dikaryotic.
- **Motile stage**– Absent.
- **Asexual spore**– Arthrospores, oidia, conidia.
- **Sexual spore**– Basidiospores.
- **Pathogenic relationship** is facultative or obligate parasite.

LIFE CYCLE OF FUNGI

The life cycle of fungi has many different patterns based on the species of the fungi. Not all fungi reproduce in the same way. While some fungi reproduce sexually, others reproduce asexually. Therefore, we are going to look at the life cycle of a fungi in asexual and sexual stage.

Sexual Reproduction of Fungi –

a. **Spore (Haploid):**

   All fungi begin their life cycle in this stage. This is the first stage in the life cycle of a fungus. In the beginning, all spores are haploid which means that they have only a single copy of their entire genetic material. These spores migrate far distances through air by grabbing on to other organisms on the way. After locating a favourable living environment, they grow a bunch of root-like structures called mycelium. Nutrients are transferred through mycelium in order for spores to develop.

b. **Mycelium (Diploid):**

   When the mycelium grows and develops, it might encounter another fungi. If the two fungi are compatible, a cell from each of the two mycelium fungi fuse together to form into another new single cell. These new fused cells are diploid as they have more than one copy of their genetic information.

c. **Meiosis:**

After the fungi has become mycelium, it enters the next process known as meiosis. During meiosis, a single cell splits into two cells and the genetic material from both parents gets mixed up. The produced two daughter cells do not have identical features to their parents and they do not look similar to each other as well.

Key Points

- Viruses are classified into four groups based on shape: filamentous, isometric (or icosahedral), enveloped, and head and tail.
- Many viruses attach to their host cells to facilitate penetration of the cell membrane, allowing their replication inside the cell.
- Non-enveloped viruses can be more resistant to changes in temperature, pH, and some disinfectants than are enveloped viruses.
- The virus core contains the small single- or double-stranded genome that encodes the proteins that the virus cannot get from the host cell.

Key Terms

- **capsid**: the outer protein shell of a virus
- **envelope**: an enclosing structure or cover, such as a membrane
- **filamentous**: Having the form of threads or filaments
- **isometric**: of, or being a geometric system of three equal axes lying at right angles to each other (especially in crystallography)

WHAT IS THE BASE STRUCTURE OF A VIRUS?

- The simplest virions consist of two basic components: **nucleic acid (single- or double-stranded RNA or DNA) and a protein coat**, the capsid, which functions as a shell to protect the viral genome from nucleases and which during infection attaches the virion to specific receptors exposed on the prospective host cell.

WHAT 3 MAIN STRUCTURES MAKE UP A TYPICAL VIRUS?

- Viruses of all shapes and sizes consist of **a nucleic acid core, an outer protein coating or capsid, and sometimes an outer envelope**.

IS TMV A HELICAL VIRUS?

- Tobacco mosaic virus (TMV; Tobamovirus, Virgaviridae) is a rodlike virus with a length of 300 nm and diameter of 18 nm. TMV capsids are composed of 2130 identical protein subunits, which assemble around the viral ssRNA to form a **helical structure**, with a hollow central cavity of 4 nm diameter.

VIRAL MORPHOLOGY

Viruses are acellular, meaning they are biological entities that do not have a cellular structure. Therefore, they lack most of the components of cells, such as organelles, ribosomes, and the plasma membrane.

- A virion consists of a nucleic acid core, an outer protein coating or capsid, and sometimes an outer envelope made of protein and phospholipid membranes derived from the host cell.
- The capsid is made up of protein subunits called capsomeres. Viruses may also contain additional proteins, such as enzymes.
- The most obvious difference between members of viral families is their morphology, which is quite diverse.
- An interesting feature of viral complexity is that host and virion complexity are uncorrelated. Some of the most intricate virion structures are observed in bacteriophages, viruses that infect the simplest living organisms: bacteria.

MORPHOLOGY

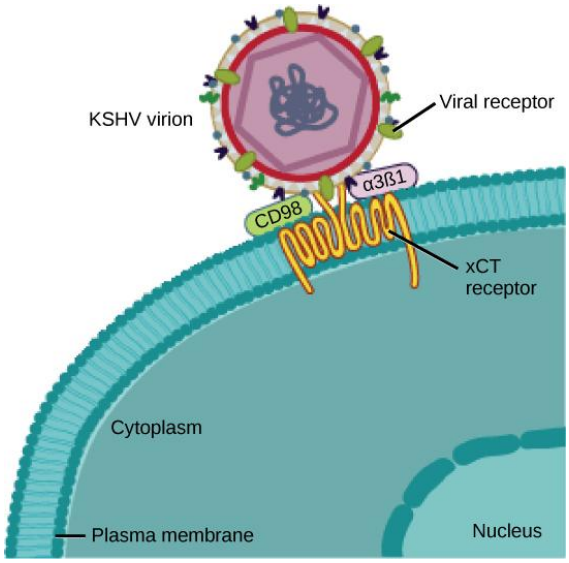


Figure: **Example of a virus attaching to its host cell**:

- The KSHV virus binds the xCT receptor on the surface of human cells. This attachment allows for later penetration of the cell membrane and replication inside the cell.

- Viruses come in many shapes and sizes, but these are consistent and distinct for each viral family.
- In general, the shapes of viruses are classified into four groups: filamentous, isometric (or icosahedral), enveloped, and head and tail. Filamentous viruses are long and cylindrical. Many plant viruses are filamentous, including TMV (tobacco mosaic virus).

- Isometric viruses have shapes that are roughly spherical, such as poliovirus or herpesviruses. Enveloped viruses have membranes surrounding capsids. Animal viruses, such as HIV, are frequently enveloped. Head and tail viruses infect bacteria.
- They have a head that is similar to icosahedral viruses and a tail shape like filamentous viruses.
- Many viruses use some sort of glycoprotein to attach to their host cells via molecules on the cell called viral receptors.
- For these viruses, attachment is a requirement for later penetration of the cell membrane, allowing them to complete their replication inside the cell.
- The receptors that viruses use are molecules that are normally found on cell surfaces and have their own physiological functions.
- Viruses have simply evolved to make use of these molecules for their own replication.
- Overall, the shape of the virion and the presence or absence of an envelope tell us little about what disease the virus may cause or what species it might infect, but they are still useful means to begin viral classification.
- Among the most complex virions known, the T4 bacteriophage, which infects the *Escherichia coli* bacterium, has a tail structure that the virus uses to attach to host cells and a head structure that houses its DNA.
- Adenovirus, a non-enveloped animal virus that causes respiratory illnesses in humans, uses glycoprotein spikes protruding from its capsomeres to attach to host cells.
- Non-enveloped viruses also include those that cause polio (poliovirus), plantar warts (papillomavirus), and hepatitis A (hepatitis A virus).

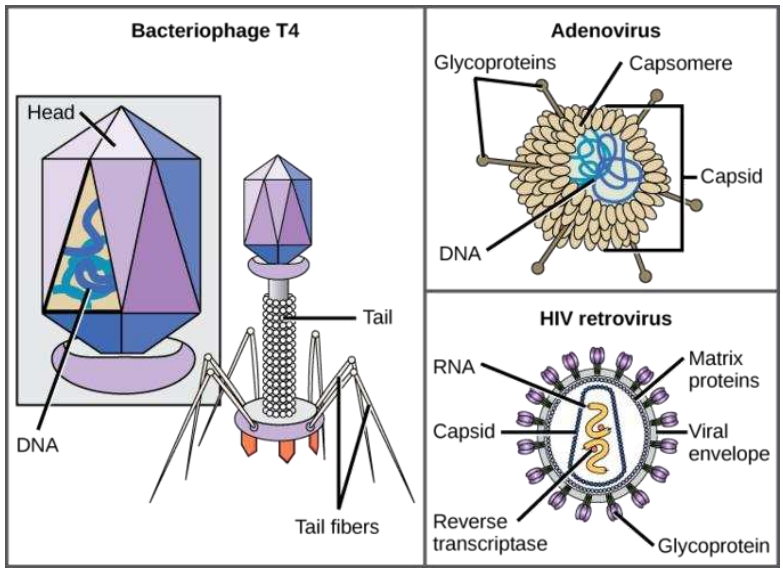


Figure: **Examples of virus shapes**:

- Viruses can be either complex in shape or relatively simple.

- This figure shows three relatively-complex virions: the bacteriophage T4, with its DNA-containing head group and tail fibers that attach to host cells; adenovirus, which uses spikes from its capsid to bind to host cells; and HIV, which uses glycoproteins embedded in its envelope to bind to host cells.

- Enveloped virions like HIV consist of nucleic acid and capsid proteins surrounded by a phospholipid bilayer envelope and its associated proteins.
- Glycoproteins embedded in the viral envelope are used to attach to host cells. Other envelope proteins include the matrix proteins that stabilize the envelope and often play a role in the assembly of progeny virions.
- Chicken pox, influenza, and mumps are examples of diseases caused by viruses with envelopes. Because of the fragility of the envelope, non-enveloped viruses are more resistant to changes in temperature, pH, and some disinfectants than are enveloped viruses.

VIRUS STRUCTURE

Viruses are not plants, animals, or bacteria, but they are the quintessential parasites of the living kingdoms. Although they may seem like living organisms because of their prodigious reproductive abilities, viruses are not living organisms in the strict sense of the word.

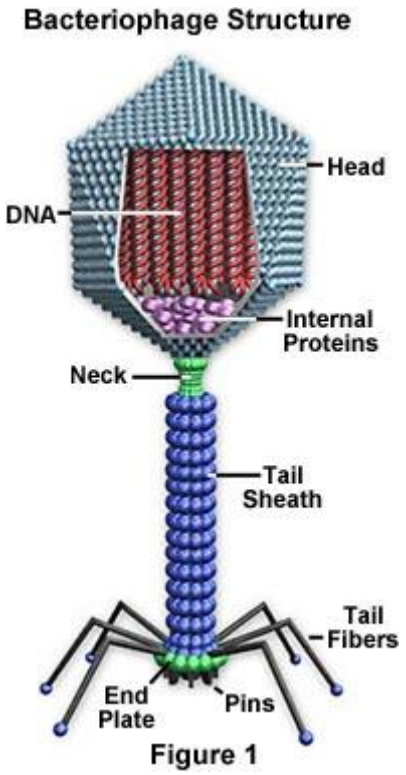


Figure 1

- Without a host cell, viruses cannot carry out their life-sustaining functions or reproduce. They cannot synthesize proteins, because they lack ribosomes and must use the ribosomes of their host cells to translate viral messenger RNA into viral proteins.
- Viruses cannot generate or store energy in the form of adenosine triphosphate (ATP), but have to derive their energy, and all other metabolic functions, from the host cell.
- They also parasitize the cell for basic building materials, such as amino acids, nucleotides, and lipids (fats).
- Although viruses have been speculated as being a form of protolife, their inability to survive without living organisms makes it highly unlikely that they preceded cellular life during the Earth's early evolution.
- Some scientists speculate that viruses started as rogue segments of genetic code that adapted to a parasitic existence.
- All viruses contain nucleic acid, either DNA or RNA (but not both), and a protein coat, which encases the nucleic acid.
- Some viruses are also enclosed by an envelope of fat and protein molecules. In its infective form, outside the cell, a virus particle is called a virion.
- Each virion contains at least one unique protein synthesized by specific genes in its nucleic acid. Viroids (meaning "viruslike") are disease-causing organisms that contain only nucleic acid and have no structural proteins.
- Other viruslike particles called prions are composed primarily of a protein tightly integrated with a small nucleic acid molecule.
- Viruses are generally classified by the organisms they infect, animals, plants, or bacteria. Since viruses cannot penetrate plant cell walls, virtually all plant viruses are transmitted by insects or other organisms that feed on plants.
- Certain bacterial viruses, such as the T4 bacteriophage, have evolved an elaborate process of infection. The virus has a "tail" which it attaches to the bacterium surface by means of proteinaceous "pins.
- " The tail contracts and the tail plug penetrates the cell wall and underlying membrane, injecting the viral nucleic acids into the cell.
- Viruses are further classified into families and genera based on three structural considerations: 1) the type and size of their nucleic acid, 2) the size and shape of the capsid, and 3) whether they have a lipid envelope surrounding the nucleocapsid (the capsid enclosed nucleic acid).
- There are predominantly two kinds of shapes found amongst viruses: rods, or filaments, and spheres.
- The rod shape is due to the linear array of the nucleic acid and the protein subunits making up the capsid.
- The sphere shape is actually a 20-sided polygon (icosahedron).
- The nature of viruses wasn't understood until the twentieth century, but their effects had been observed for centuries.

- British physician Edward Jenner even discovered the principle of inoculation in the late eighteenth century, after he observed that people who contracted the mild cowpox disease were generally immune to the deadlier smallpox disease.
- By the late nineteenth century, scientists knew that some agent was causing a disease of tobacco plants, but would not grow on an artificial medium (like bacteria) and was too small to be seen through a light microscope.
- Advances in live cell culture and microscopy in the twentieth century eventually allowed scientists to identify viruses. Advances in genetics dramatically improved the identification process.

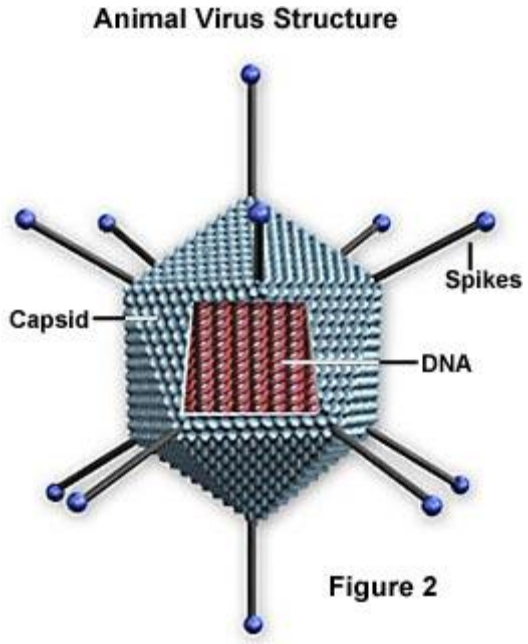


Figure 2

- **Capsid** - The capsid is the protein shell that encloses the nucleic acid; with its enclosed nucleic acid, it is called the nucleocapsid. This shell is composed of protein organized in subunits known as capsomers. They are closely associated with the nucleic acid and reflect its configuration, either a rod-shaped helix or a polygon-shaped sphere. The capsid has three functions: 1) it protects the nucleic acid from digestion by enzymes, 2) contains special sites on its surface that allow the virion to attach to a host cell, and 3) provides proteins that enable the virion to penetrate the host cell membrane and, in some cases, to inject the infectious nucleic acid into the cell's cytoplasm. Under the right conditions, viral RNA in a liquid suspension of protein molecules will self-assemble a capsid to become a functional and infectious virus.
- **Envelope** - Many types of virus have a glycoprotein envelope surrounding the nucleocapsid. The envelope is composed of two lipid layers interspersed with protein molecules (lipoprotein bilayer) and may contain material from the membrane of a host cell as well as that of viral origin. The virus obtains the lipid molecules from the cell

membrane during the viral budding process. However, the virus replaces the proteins in the cell membrane with its own proteins, creating a hybrid structure of cell-derived lipids and virus-derived proteins. Many viruses also develop spikes made of glycoprotein on their envelopes that help them to attach to specific cell surfaces.

- **Nucleic Acid** - Just as in cells, the nucleic acid of each virus encodes the genetic information for the synthesis of all proteins. While the double-stranded DNA is responsible for this in prokaryotic and eukaryotic cells, only a few groups of viruses use DNA. Most viruses maintain all their genetic information with the single-stranded RNA. There are two types of RNA-based viruses. In most, the genomic RNA is termed a plus strand because it acts as messenger RNA for direct synthesis (translation) of viral protein. A few, however, have negative strands of RNA. In these cases, the virion has an enzyme, called RNA-dependent RNA polymerase (transcriptase), which must first catalyze the production of complementary messenger RNA from the virion genomic RNA before viral protein synthesis can occur.

Viruses are classified into four groups based on shape: **filamentous, isometric (or icosahedral), enveloped, and head and tail**. Many viruses attach to their host cells to facilitate penetration of the cell membrane, allowing their replication inside the cell.

WHAT IS VIRUS AND ITS STRUCTURE?

All viruses contain nucleic acid, either DNA or RNA (but not both), and **a protein coat**, which encases the nucleic acid. Some viruses are also enclosed by an envelope of fat and protein molecules. In its infective form, outside the cell, a virus particle is called a virion.

WHAT IS THE SHAPE OF A VIRUS?

Most viruses have **icosahedral or helical capsid structure**, although a few have complex virion architecture. An icosahedron is a geometric shape with 20 sides, each composed of an equilateral triangle, and icosahedral viruses increase the number of structural units in each face to expand capsid size.

WHAT ARE 5 CHARACTERISTICS OF VIRUSES?

These are: 1) attachment; 2) penetration; 3) uncoating; 4) replication; 5) assembly; 6)release. As shown in , the virus must first attach itself to the host cell.

WHICH OF THE 7 CHARACTERISTICS OF LIFE DO VIRUSES HAVE?

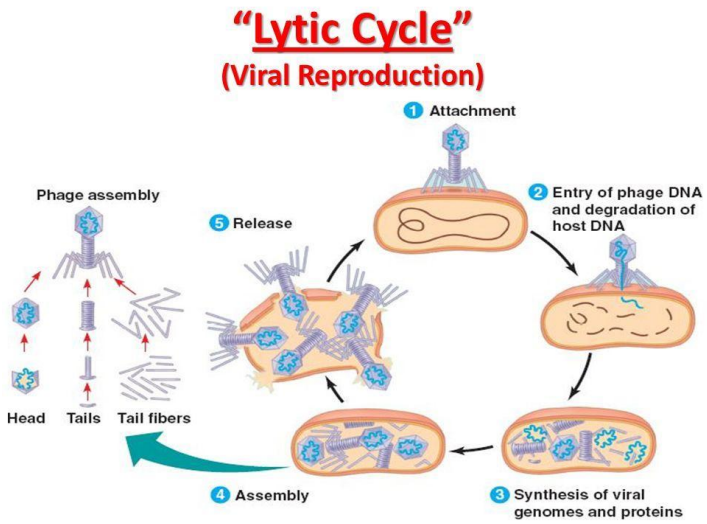
According to the seven characteristics of life, all living beings must be able to respond to stimuli; **grow over time; produce offspring**; maintain a stable body temperature; metabolize energy; consist of one or more cells; and adapt to their environment.
How do viruses and bacteria enter the body?

Microorganisms capable of causing disease—or pathogens—usually enter our bodies **through the eyes, mouth, nose, or urogenital openings**, or through wounds or bites that breach the skin barrier. Organisms can spread, or be transmitted, by several routes.

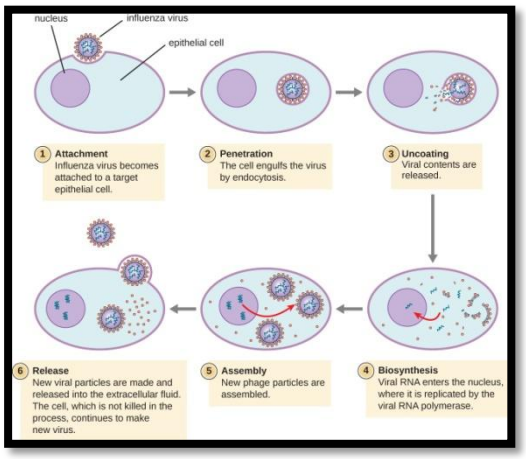
VIRAL REPRODUCTION—A LYTIC INFECTION

Let's look at what happens when a virus attacks our cells. Most viruses reproduce through a process called lytic infection.

During lytic infection, a virus enters the host cell, makes a copy of itself, and causes the cell to burst, or lyse. I



LIFE CYCLE OF VIRUSES

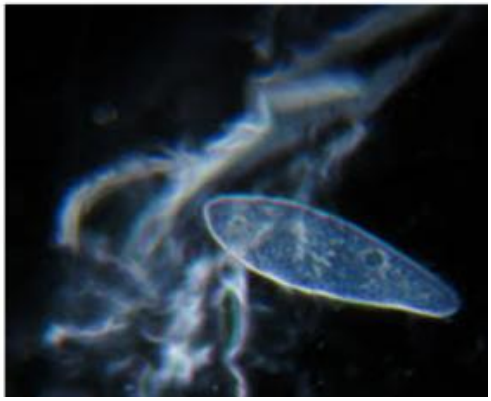


PROTOZOA

What is Protozoa?

- In the sub-kingdom kingdom Protista lays the Protozoa.
- Also, Protozoa are found in every possible habitat of the earth. Protozoa is a single cell animal that we can find in every possible habitat on earth.
- Furthermore, the scientist has described more than 50 thousand species of Protozoa. Moreover, they are herbivores, carnivores, and omnivores.
- Most noteworthy, most of the diseases related to Protozoa happen inside the patient of AIDS (Acquired Immune Deficiency Syndrome).

Examples: Rhizopoda, Sarcodina, amoeba, mycetozoa, etc.



STRUCTURE OF PROTOZOA

- Protozoa are eukaryotic cells.
- They are unicellular organisms.
- Their size ranges from 1 micrometer to 200000 or may be up to 200000 micrometres in diameter.
- The size of smaller protozoa is from 1 to 10 μm long.
- They contain membrane bounded organelles in their cytoplasm such as ribosome, Golgi apparatus.
- Protozoa contain a well organised nucleus which is covered with membrane.
- The types of organelles present in protozoa vary from species to species. They contain some characteristic organelles such as the Trichocysts of Paramecium, certain skeletal structures, Contractile vacuoles.
- The protozoa contain a vesicular nucleus. As such, the chromatic is scattered, the nucleus resulting a diffuse in look.

- ❖ The vesicular nucleus of Phylum *Apicomplexa* contains one or more nucleoli with DNA whereas the DNA is absent in the endosome of trypanosomes.
- ❖ They contain pseudopodia, flagella and cilia which help them in locomotion. These locomotory structures are covered by the plasma membrane.
- ❖ Some protozoa also contain a rigid structure known as pellicle which gives them shape and also helps in twisting and bending during locomotion.

LIFE CYCLE

Protozoa pass through different stages of life throughout its life. Also, the stages differ in activity and structure. In addition, their life cycle revolves around feeding, multiplying (reproduction), growing and repeating the same process again and again.

PARASITIC PROTOZOA

The life cycle of parasitic protozoa occurs intracellular or in the lumen of given organs. Because of the diversity, different species follow different patterns of life cycle.

There is three most common pattern have been found in protozoa such as;

(i). First pattern

- This pattern is mainly found in phylum Apicomplexa. In this method the alteration occurs between asexual and sexual reproductive stages.
- The cycel is started with the asexual reproduction. In the first stage the population of host's tissue is increased by the schizogony (involving mitosis and cytokinesis).
- After that the population undergoes gametogony(a sexual process) and develops gametes.
- These gametes undergo sporogony (asexual process) and form sporozoites. These sporozoites have a capability to infect a new host cell.
- When the sporozoite entered the host cell they started the reproduction cycle again.
- Apicomplexa required two hosts(vertebrate and invertebrate) to complete their life cycle. Inside the vertebrate they undergo the schizogony and gametogony. Inside the invertebrate the gametes unite and sporogony occurs in the tissues.

(ii) Second Pattern

- It is the most common pattern among the flagellates, where asexual reproduction is involved.

- In this cycle a number of morphological transformations occur. All of them reproduced by the binary fission.
- Some of them use a vertebrate host to complete their life cycle, where they transmit from one host to another through cysts.

(iii). Third pattern

- This is mainly found in amoebas and completed through the asexual reproduction.
- It required a single host to complete their reproduction. For example; trophozoites live in the lumen of the gut where they continuously increase their number by binary fission.
- Here, under certain conditions, the trophozoites are stimulated to encyst as they undergo nuclear division within the cyst. The cycle continues, When another host ingest the cyst.

REPRODUCTION

- The process of reproduction in protozoa can be sexual as well as asexual. Moreover, the most common type of asexual reproduction is binary fission in which the organelles duplicate inside the protozoan and then divide into two new organisms (protozoa).

- Besides, Plasmodium, Apicomplexans, and Toxoplasma follow the sexual reproduction cycle that involves the production of gametes which then fertilize to form a zygote and so on.

- Most noteworthy, some protozoa have a complex life cycle that requires two host species while others require only one host in their whole life cycle. Moreover, a single protozoan has the capacity to multiply itself much fold inside the body of the host.

- But, the reproduction mechanism has limitations such as a defense mechanism of the host or death of the host. As the defense mechanism can either eliminate them or limit their production to yield the chronic infection.

NUTRITION

- Protozoa have holozoic nutrition it means that they require organic matter which can be a solution or particulate.

- Furthermore, they engulf their food or droplet with a temporary mouth after which their bodies perform the digestion and absorption of food vacuole and finally eject the waste substance.

- Besides, many protozoa have a permanent mouth for the ingestion of food. Also, they have metabolic pathways similar to higher animals that require the same type of inorganic and organic compounds.

MARUDHAR KESARI JAIN COLLEGE FOR WOMEN

VANIYAMBADI

DEPARTMENT OF BIOTECHNOLOGY

MICROBIOLOGY

II B.Sc BIOTECHNOLOGY **SUBJECT CODE FBT31**

MS.S.ANUPRIYA

UNIT -3

NUTRITIONAL REQUIREMENTS AND NUTRITIONAL GROUPING OF MICROORGANISMS

BASIC NUTRITIONAL REQUIREMENTS FOR GROWTH OF MICROORGANISMS

For growth and nutrition of bacteria, the minimum nutritional requirements are **water, a source of carbon, a source of nitrogen and some inorganic salts**. Water is the vehicle of entry of all nutrients into the cell and for the elimination of waste products.

NUTRIENT REQUIREMENT OF MICROORGANISMS

Microorganisms requires macronutrients, micronutrients and growth factors, for their growth. These nutrients help in constructing the cellular components like proteins, nucleic acids and lipids.

MINERAL NUTRIENTS:

The microbial nutrients can be classified as macro (major) nutrients, and micro (minor) nutrients or trace elements on the basis of their amount required.

1. MACRO OR MAJOR MINERAL NUTRIENTS:

- The microbial cells contain water accounting for some 80-90% of their total weight and, therefore, the water is always the major essential nutrient in quantitative terms.
- The solid matter of cells contain, in addition to oxygen and hydrogen (derivable metabolically from water), the other macro (major) elements, namely, carbon, nitrogen, phosphorus, sulphur, potassium, magnesium, sodium, calcium and iron in order of decreasing abundance.
- About 95% of cellular dry weight of microbial cells is accounted for only six macro (major) elements (O, H, C, N, P and S).
- Carbon assumes great importance as the main constituent of all organic cell materials and represents about 50% of cell's dry weight. CO_2 is the most oxidized form of carbon and the photo-synthetic microorganisms reduce CO_2 to organic cell constituents.
- On the other hand, all the non-photosynthetic microorganisms obtain their carbon requirement mainly from organic nutrients which contain reduced carbon compounds.

- These organic compounds not only provide the carbon for synthesis but also meet the energy requirement by entering into energy yielding metabolic pathways and are eventually oxidised to CO_2.
- Some microbes have the ability to synthesize all their cellular components using a single organic carbon source while others, in addition to this one major carbon source, also need other complex carbon containing components which they cannot synthesize.
- These components are called growth factors and include vitamins. Some microbes can utilize more than one carbon compound and exhibit a great degree of versatility. The others, however, are specialized in this regard.
- Sulphur and nitrogen are taken up by most organisms and are subsequently reduced within the cell and utilized in other biosynthetic processes. The sulphur and nitrogen requirements of most organisms can also be met with organic nutrients that contain these two elements in reduced organic combinations such as amino acids.
- A few microorganisms are capable of reducing elemental nitrogen to ammonia and this process of nitrogen assimilation is known as biological nitrogen fixation.
- Most of the microorganisms need molecular oxygen for respiration. In these, the oxygen serves as terminal electron acceptor, and such organisms are referred to as 'obligate aerobes'.
- As opposed to this there are a few organisms which do not use molecular oxygen as terminal electron acceptor. We recall that oxygen is a component of the cellular material of all the microorganisms. These microbes are called 'obligate anaerobes'.
- In fact, molecular oxygen is toxic to these organisms. Aerobes which can grow in the absence of oxygen are called 'facultative anaerobes' and the anaerobes which can grow in the presence of oxygen are referred to as 'facultative aerobes'.
- In addition to these major classes, there are organisms which grow best at reduced oxygen pressure but are obligate aerobes and these are called 'Microaerophilic'.
- Elements that are required in large amounts are called macronutrients. Nitrogen (N), Carbon (C), Oxygen (O), Hydrogen (H), Sulphur (S) and Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg) and Iron (Fe) are macro elements.
- Nitrogen is needed for the synthesis of amino acids, nucleotides like purines and pyrimidines which are part of nucleic acids (DNA and RNA).
- Phosphorus is a part of phospholipids, nucleotides like ATP and phosphodiester bonds of nucleic acids.
- Carbon, Hydrogen and Oxygen are the backbone of all organic macromolecules like peptidoglycan, proteins and lipids and nucleic acids.
- Sulphur is needed for the synthesis of thiamin, biotin, and aminoacids like cysteine and methionine.
- Potassium, Calcium, Magnesium and Iron exist as cations in the cell. These element plays vital role in the metabolic activity of microorganisms. Potassium (K^+) is needed for the activity of many enzymes Example: Pyruvate Kinase.
- Calcium (Ca^{2+}) is involved in the heat resistance of bacterial endospores.
- Magnesium (Mg^{2+}) binds with ATP and serves as a cofactor of enzymes like hexokinase.

- Iron (Fe^{2+} or Fe^{3+}) is present in cytochromes and act as cofactors for cytochrome oxidase, catalase and peroxidase.

2. MICRO OR MINOR MINERAL NUTRIENTS OR TRACE ELEMENTS:

- Nutrients that are needed in trace quantities are called micronutrients. Example: Zinc (Zn), Molybdenum (Mo), Cobalt (Co), Manganese (Mn).
- Besides macro and micronutrients, some microorganisms need growth factors like amino acids, purines and pyrimidines and vitamins. Example: Biotin is required by *Leuconostoc* sp and folic acid is required by *Enterococcus faecalis*.
- The microorganisms, in general do not use only macro (major) elements but also others like cobalt, copper, manganese, molybdenum, nickel, selenium, tungsten, vanadium and zinc which are required in residual fraction by nearly all microorganisms.
- These elements are often referred to as minor (micro) nutrients or trace elements. The micronutrients or trace elements are nevertheless just as critical to cell function as are the macronutrients.

NUTRITIONAL TYPES OF MICROORGANISMS

- Phototrophs – Energy for growth is derived from sunlight.
- Chemotrophs – Energy for growth is derived from the oxidation of either organic and inorganic chemical compounds.
- Lithotrophs : Source of electrons is reduced inorganic compounds

Nutritional class	Energy/Electron/Carbon source	Organisms
Photoautotrophs	Light energy Inorganic e^- donor CO_2	Cyanobacteria, Purple and Green sulphur Bacteria
Photoheterotrophs	Light energy Organic e^- donor Organic carbon source	Purple and Green Nonsulfur bacteria
Chemoautotrophs	Inorganic chemical compounds as energy source Inorganic e^- donor CO_2	Nitrifying bacteria, Iron bacteria
Chemoheterotrophs	Organic compounds as energy, electron and carbon source.	Most pathogenic bacteria, fungi and protozoa.

BACTERIAL NUTRITION

- The bacterial cell has the same general chemical pattern as the cells of other organisms. The bacterial cell contains water (80% of total weight), proteins, polysaccharides, lipids, nucleic acids, mucopeptides and low molecular weight compounds.
- For growth and nutrition of bacteria, the minimum nutritional requirements are water, a source of carbon, a source of nitrogen and some inorganic salts.
- Water is the vehicle of entry of all nutrients into the cell and for the elimination of waste products.

- Bacteria can be classified nutritionally based on their energy requirements and on their ability to synthesise essential metabolites.
- Bacteria which derive energy from sunlight are called phototrophs. Those that obtain energy from chemical reactions are called chemotrophs. Bacteria that can synthesise all their organic compounds are called autotrophs.
- They are able to use atmospheric carbon dioxide and nitrogen. They are capable of independent existence in water and soil. They are of no medical importance. Some bacteria are unable to synthesise their own metabolites. They depend on preformed organic compounds. They are called heterotrophs. These bacteria are unable to grow with carbon dioxide as the sole source of carbon.
- Their nutritional requirements vary widely. Some may require only a single organic substance like glucose. Others may need a large number of different compounds like amino acids, nucleotides, lipids, carbohydrates and coenzymes. Bacteria require a supply of inorganic salts.
- They require anions like phosphate and sulphate anions and cations like sodium, potassium, magnesium, iron and calcium. Some ions like cobalt may be required in trace amounts. Some bacteria require certain organic compounds in minute quantities.
- These are called growth factors or bacterial vitamins. Growth factors are called essential when growth does not occur in their absence. Accessory growth factors are those which enhance growth without being absolutely necessary for it.
- In many cases, bacterial vitamins are same as vitamins necessary for nutrition of mammals, for example, B group vitamins – thiamine, riboflavin, pyridoxine, nicotinic acid, folic acid and vitamin B12

GASEOUS REQUIREMENTS

- Depending on the influence of oxygen on growth and survival, bacteria are divided into aerobes and anaerobes. Aerobic bacteria require oxygen for growth.
- They may be obligate aerobes or facultative anaerobes. Obligate aerobes grow only in the presence of oxygen, for eg. *Cholera bacillus.*
- Facultative anaerobes are ordinarily aerobic but can grow in the absence of oxygen, though less abundantly. Most bacteria of medical importance are facultative anaerobes. Anaerobic bacteria, such as clostridia grow in the absence of oxygen. Obligate anaerobes may even die on exposure to oxygen. Microaerophilic bacteria are those that grow best in the presence of low oxygen tension.
- In case of aerobes, atmospheric oxygen is the final electron acceptor in the process of respiration (aerobic respiration). In this case, the carbon and energy source may be completely oxidised to carbon dioxide and water.
- Energy is provided by the production of energy-rich phosphate bonds and the conversion of adenosine diphosphate (ADP) to adenosine triphosphate (ATP). This process is called oxidative phosphorylation. Anaerobic bacteria use compounds like nitrates or sulphates instead of oxygen as final electron acceptors in the process of respiration (anaerobic respiration).
- A more common process used by these bacteria in anaerobic metabolism is fermentation. It is defined as the process by which complex organic compounds, such

as glucose, are broken down by the action of enzymes into simpler compounds without the use of oxygen.

- This process leads to the formation of several organic end products such as organic acids and alcohols, as well as of gas (carbon dioxide and hydrogen). For example, Escherichia coli ferments glucose with the production of acid and gas. It also ferments lactose.
- During the process of fermentation, energy-rich phosphate bonds are produced by the introduction of organic phosphate into intermediate metabolites. This process is known as substrate-level phosphorylation. The energy-rich phosphate groups so formed are used for conversion of ADP to ATP. All bacteria require some amounts of carbon dioxide for growth.
- This is obtained from the atmosphere or from the cellular metabolism of the bacterial cell. Some bacteria like Brucella abortus require much higher levels of carbon dioxide (5- 10%) for growth. They are called capnophilic.

SELECTIVE AND DIFFERENTIAL MEDIA

WHAT IS THE MAJOR DIFFERENCE BETWEEN DIFFERENTIAL AND SELECTIVE MEDIA?

The key difference between selective media and differential media is that **selective media are used to grow and isolate a specific type of microorganism by suppressing the growth of other microorganisms** while differential media are used to visually distinguish microorganisms from one another.

MAIN DIFFERENCE – SELECTIVE VS DIFFERENTIAL MEDIA

- Selective and differential media are two types of media used to isolate and identify microorganisms.
- The **main difference** between selective and differential media is that **selective media are used to isolate a particular strain of microorganisms whereas differential media are used to identify and differentiate a closely-related group of microorganisms**.
- Selective media use specific growth characteristics of a microorganism to selectively grow that microorganism in the growth medium.
- Differential media allow the characterization of several microorganisms based on the growth patterns of them.

WHAT IS SELECTIVE MEDIA?

- Selective media refer to a type of growth media that allows the growth of selected microorganisms in the medium.
- For example, if a particular microorganism is resistant to a particular antibiotic such as tetracycline or ampicillin, that antibiotic can be added to the medium, prohibiting the growth of other microorganisms in that medium.
- Selective growth media also ensures the survival and proliferation of microorganisms with certain properties.
- The gene that gives the ability to grow in the selective medium is known as the marker.

- Eukaryotic cells can also be grown in selective media. The selective media for eukaryotic cells commonly contain neomycin. Some examples of selective media are shown in the table below.

TYPES OF SELECTIVE MEDIA

Selective Media	Type of Organisms
Eosin methylene blue (EMB)	Gram-negative bacteria
YM	Yeast and mold
MacConkey agar	Gram-negative bacteria
Hektoen enteric agar (HE)	Gram-negative bacteria
Mannitol salt agar (MSA)	Gram-positive bacteria
Terrific Broth (TB)	Recombinant strains of *E.coli*
Xylose lysine desoxycholate (XLD)	Gram-negative bacteria

WHAT IS DIFFERENTIAL MEDIA

- Differential media refer to a type of growth media that allows the differentiation of closely-related microorganisms.
- Differential media is also known as **indicator media**.
- The biochemical characteristics of a microorganism are used in the differentiation and identification of the microorganism among other microorganisms.
- Specific nutrients or indicators are used in the differential media.
- The indicators can be either eosin y, neutral red, phenol red or methylene blue.
- They are added to the medium to indicate the above-mentioned characteristics visible in the medium.

TYPES OF DIFFERENTIAL MEDIA

Differential Media	Significance
Blood agar	Contains bovine heart blood that becomes transparent in the presence of hemolytic
Streptococcuseosin methylene blue (EMB)	Differential for lactose and sucrose fermentation

MacConkey (MCK)	Differential for lactose fermentation
Mannitol salt agar (MSA)	Differential for mannitol fermentation
X-gal plates	Differential for lac operon mutants

SIMILARITIES BETWEEN SELECTIVE AND DIFFERENTIAL MEDIA

- Selective and differential media are two types of growth media used to grow microorganisms.
- Both selective and differential media are used to isolate and identify microorganisms.

DIFFERENCE BETWEEN SELECTIVE AND DIFFERENTIAL MEDIA

DEFINITION

Selective Media:
Selective media refer to a type of growth media that allows the growth of selected microorganisms in the medium.
Differential Media:
Differential media refer to a type of growth media that allows the differentiation of closely-related microorganisms.

PURPOSE

Selective Media:
Selective media are used to isolate a particular strain of microorganisms.
Differential Media:
Differential media are used to identify and differentiate closely-related microorganisms.

TYPES OF CHARACTERISTICS

Selective Media:
Selective media use specific growth characteristics of a particular microorganism to select it from the others.
Differential Media:
Differential media use unique growth patterns of microorganisms to differentiate them from others.

NUMBER OF MICROORGANISMS

Selective Media:
Selective media only allow the growth of a single microorganism in the medium.
Differential Media:

Differential media allow several closely-related microorganisms to grow in the medium.

INDICATORS

Selective Media:
Selective media do not use indicators.
Differential Media:
Differential media use indicators.

CONCLUSION

- Selective and differential media are two types of growth media used to isolate and identify microorganisms.
- Selective media are used to isolate a particular type of microorganism by giving a specific condition for the growth of that particular microorganism.
- Differential media are used to identify and differentiate microorganisms from a closely-related group with the help of unique growth patterns.
- The main difference between selective and differential media is their role in the identification of microorganisms.

ENRICHMENT MEDIA

WHAT IS THE DEFINITION OF ENRICHMENT MEDIA?

Enrichment media is **a media that allows only the growth of a particular type of microorganism**. They do not contain any inhibitors to inhibit the growth of other organisms.The enriched media is used to grow nutritionally exacting fastidious microorganisms.

WHAT IS ENRICHMENT MEDIA GIVE EXAMPLE?

Enrichment media are liquid media. The examples of enriched media are **blood agar, chocolate agar, Loeffler's serum**, etc. The examples of enrichment media are Selenite F broth, tetrathionate broth, alkaline peptone water (APW), etc.

WHAT IS THE PURPOSE OF ENRICHMENT MEDIA?

Enriched media contain **the nutrients required to support the growth of a wide variety of organisms, including some of the more fastidious ones**.

WHAT IS THE PURPOSE OF PRE ENRICHMENT IN MICROBIOLOGY?

The purpose of preenrichment is **to resuscitate low levels of injured Salmonella and to allow them to proliferate to detectable levels**

WHAT IS THE DIFFERENCE BETWEEN ENRICHED MEDIA AND ENRICHMENT MEDIA?

Growth medium or Culture Medium is a sterilized medium that contains substances required for the growth of microorganisms such as bacteria, protozoans, algae and fungi. The growth

medium may be solid, semisolid or liquid. It usually consists of complex ingredients such as peptone, meat extract, yeast extract, inorganic salts and organic compounds. It is prepared according to the requirement for organism growth. The growth media are of different types.

COMPLETE EXPLANATION:

Enriched media	Enrichment media
Enriched media means they contain all the nutrients required for the growth of a wide variety of organisms.	Enrichment media is a media that allows only the growth of a particular type of microorganism.
They do not contain any inhibitors to inhibit the growth of other organisms.	Inhibitors are usually added to enrichment media to stop the growth of unwanted organisms.
They are usually in the solidified form.	They are usually in liquid form.
The enriched media is used to grow nutritionally exacting fastidious microorganisms.	The enrichment media inhibit the growth of unwanted commensal and contaminating bacterias.
The extra nutrients added to the enriched media are blood, serum, egg yolk etc	Some of the inhibitors added to enrichment media are antibiotics, dyes, chemicals, pH alteration etc.
Examples include blood agar, mannitol Salt Agar and chocolate agar.	Examples include Lowenstein Jensen medium, selenite F broth, tetrathionate broth, alkaline peptone water, pseudosel Agar etc.

MICROBIAL ASSAY MEDIUM

MICROBIOLOGICAL ASSAY CULTURE MEDIUM.

- A microbiological assay culture medium is a device that consists primarily of liquid or solid biological materials intended for medical purposes to cultivate selected test microorganisms in order to measure by microbiological procedures the concentration in a patient's serum of certain substances, such as amino acids, antimicrobial agents, and vitamins.
- The concentration of these substances is measured by their ability to promote or inhibit the growth of the test organism in the innoculated medium. Test results aid in the diagnosis of disease resulting from either deficient or excessive amounts of these substances in a patient's serum. Tests results may also be used to monitor the effects of the administration of certain antimicrobial drugs.

WHAT IS AN ASSAY IN MICROBIOLOGY?

A microbiological assay defined as **qualitative or quantitative determination of chemical compound from a simple or even complex material with the use of microorganisms**.

WHY DO WE USE 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**

WHAT ARE THE METHODS OF MICROBIOLOGICAL ASSAY?

There are three main methods of microbiological assay by which the potency of samples and standard solution may be compared: **(1) dilution methods, (2) turbidimetric, titrimetric, and gravimetric methods, and (3) diffusion methods**. Microbiological assays are called dilution assays.

WHAT ARE THE ADVANTAGES OF MICROBIAL ASSAY?

A primary advantage of microbiological methods is **the ability to assay complete food matrices at naturally .**This characteristic often makes a microbiological procedure the method of choice when looking at low natural levels (nonfortified) in a food.

GROWTH CURVE

- When fresh liquid medium is inoculated with a given number of bacteria and incubated for sufficient period of time, it gives a characteristic growth pattern of bacteria.
- If the bacterial population is measured periodically and log of number of viable bacteria is plotted in a graph against time, it gives a characteristic growth curve which is known as growth curve.
- Measuring the growth rate of bacteria is a fundamental microbiological technique, and has widespread use in basic research as well as in agricultural and industrial applications.

PRINCIPLE OF BACTERIAL GROWTH CURVE

When bacteria are inoculated into a liquid medium and the cell population is counted at intervals, it is possible to plot a typical bacterial growth curve that shows the growth of cells over time.

It shows four distinct phases of growth.

- Lag phase: Slow growth or lack of growth due to physiological adaptation of cells to culture conditions or dilution of exoenzymes due to initial low cell densities.
- Log or exponential phase: Optimal growth rates, during which cell numbers double at discrete time intervals known as the mean generation time.

- ❖ Stationary phase: Growth (cell division) and death of cells counterbalance each other resulting in no net increase in cell numbers. The reduced growth rate is usually due to a lack of nutrients and/or a buildup of toxic waste constituents.
- ❖ Decline or death phase: Death rate exceeds growth rate resulting in a net loss of viable cells.

Turbidimetric determination is useful for plotting growth curves of bacteria in broth or liquid media. It is one of the simplest methods used to analyze trends in growth because it uses a spectrophotometer to track changes in the optical density (OD) over time. In other words, as the number of cells in a sample increase, the transmission of light through the sample will decrease.

MATERIALS REQUIRED FOR BACTERIAL GROWTH CURVE

- ❖ Bacterial culture (E. coli), Broth (Luria Bertani (LB) Broth, Nutrient Broth) Glass wares: Conical flasks, Measuring cylinder, Sterile test tubes, Sterile Petriplates
- ❖ Reagents: Distilled water
- ❖ Other requirements: Incubator, Shaker, Spectrophotometer, Micropipettes, Tips, Sterile Loops

PROCEDURE OF BACTERIAL GROWTH CURVE

Day 1: 1. Using sterile loop, streak a loopful of bacterial culture onto the agar plate. 2. Incubate at 37oC for 18-24 hours.

Day 2: 1. Pick up a single colony of each strain from the agar plate and inoculate it into a test tube containing 10 ml of autoclaved broth. 2. Incubate the test tube overnight at 37oC.

Day 3:

1. Take 250 ml of autoclaved broth in a sterile 500 ml conical flask.

2. Inoculate 5 ml of the overnight grown culture in above flask.

3. Take OD at zero hour. Incubate the flask at 37oC.

4. Aliquot 1 ml of the culture suspension at an interval of every 30 minutes and take the optical density (OD) at a wavelength of 600 nm using spectrophotometer, till the reading becomes static. Alternatively, 50-100 µl of formaldehyde can be added to all the 1 ml aliquots of culture suspension taken after every 30 minutes. Optical density of all the aliquots can be taken at the end of the experiment.

5. At the end of experiment, plot a graph of time in minutes on X axis versus optical density at 600nm on Y axis to obtain a growth curve of bacteria.

EXPECTED RESULT OF BACTERIAL GROWTH CURVE

A logarithmic growth curve is obtained showing the changes in size of a bacterial population over time in the culture. The growth curve is hyperbolic due to exponential bacterial growth pattern.

AXENIC CULTURE

WHAT DO YOU MEAN BY AXENIC CULTURE?

DEFINITION.

A microbial culture that contains only one species, variety, or strain of microorganism. **Supplement**. **Microorganisms can be grown under controlled laboratory conditions in culture**s where they are allowed to grow and reproduce.

WHY IS AXENIC CULTURE IMPORTANT?

In biology, axenic describes the state of a culture in which only a single species, variety, or strain of organism is present and entirely free of all other contaminating organisms. Axenic culture is **an important tool for the study of symbiotic and parasitic organisms in a controlled environment**.

PREPARATION

- Axenic cultures of microorganisms are typically prepared by subculture of an existing mixed culture. This may involve use of a dilution series, in which a culture is successively diluted to the point where subsamples of it contain only a few individual organisms, ideally only a single individual (in the case of an asexual species).
- These subcultures are allowed to grow until the identity of their constituent organisms can be ascertained. Selection of those cultures consisting solely of the desired organism produces the axenic culture. Subculture selection may also involve manually sampling the target organism from an uncontaminated growth front in an otherwise mixed culture, and using this as an inoculum source for the subculture.
- Axenic cultures are usually checked routinely to ensure that they remain axenic. One standard approach with microorganisms is to spread a sample of the culture onto an agar plate, and to incubate this for a fixed period of time.
- The agar should be an enriched medium that will support the growth of common "contaminating" organisms. Such "contaminating" organisms will grow on the plate during this period, identifying cultures that are no longer axenic

EXPERIMENTAL USE

- As axenic cultures are derived from very few organisms, or even a single individual, they are useful because the organisms present within them share a relatively narrow gene pool.
- In the case of an asexual species derived from a single individual, the resulting culture should consist of identical organisms (though processes such as mutation and horizontal gene transfer may introduce a degree of variability).

SYNCHRONOUS CULTURE

DEFINITION

A synchronous or synchronized culture is a microbiological culture or a cell culture which contains the cells that are all in the same growth stage.

- The Synchronous culture also known as the synchronous growth.
- The synchronous growth of a bacterial culture means all the cells of the culture remain at the same stage of growth and they grow all together from one phase to another.
- A population can be synchronized by manipulating their physical environments or physical composition of the medium.
- If we keep an organism under unfavourable conditions there they will metabolize very slowly, but will not divide. If we keep the organisms under favourable conditions, then all cells undergo division and stay at the same phase.
- The cells of the synchronously growing culture divide at a time, their growth curve forms a Zig Zag pattern.
- The easiest way to synchronize bacterial growth is to add some cytostatic agents so that cells don't divide and they all maintain the same state of metabolism and cell cycle. When the cytostatic agent is removed, all cells start to divide at the same time.
- Synchronous culture/Synchronous growth of bacteria consists of two phases such as stationary phase and exponential phase.

OBTAINING SYNCHRONOUS CULTURE

There are present different method for obtaining a Synchronous culture such as;

- To arrest the growth of all cells in the culture, alter the External conditions, and then again alter the conditions to continue the growth. As a result of this, all the fresh growing cells are now beginning to grow at the same stage, and they are synchronized.
- Synchronous culture can be obtained by eliminating the essential nutrient from the growth medium and later re-introduce it.
- The chemical growth inhibitors can be used to stop cell growth. When the growth is completely stopped for all cells, then remove the inhibitor from the culture and the cells will begin to grow synchronously. For example, Nocodazole is used in biological research for synchronization.
- Cells in various growth stages contain distinct physical properties. Cells in culture can thus be physically divided depending on their density or size, for instance. This can be done by using centrifugation (for density) or filtration (for size).
- The Helmstetter-Cummings technique can be used to obtain a Synchronous culture. In this method, the bacterial culture is passed through a membrane. Some of these cells will remain bound to the membrane.
- After that a fresh medium is added to the membrane, as result, the membrane-bound bacteria will start to grow. Fresh bacteria that separated from the membrane are now all at the identical stage of growth; they are accumulated in a flask that now harbors as a synchronous culture.

APPLICATION OF SYNCHRONOUS CULTURE

- Synchronous culture helps in the separation of the smallest cells from an exponentially growing culture.
- In the laboratory it is used to study the cell cycle.
- Important in the study of genetics and metabolism.

CONTINUOUS CULTURE

WHAT DOES CONTINUOUS CULTURE MEAN?

- Continuous culture is **a set of techniques used to reproducibly cultivate microorganisms at submaximal growth rates at different growth limitations** in such a way that the culture conditions remain virtually constant (in 'steady state') over extended periods of time.
- A technique used to grow microorganisms or cells continually in a particular phase of growth. For example, if a constant supply of cells is required, a cell culture maintained in the log phase is best; the conditions must therefore be continually monitored and adjusted accordingly so that the cells do not enter the stationary phase (see bacterial growth curve).
- Growth may also have to be maintained in a particular growth phase if an enzyme or chemical product is produced only during that phase.

WHAT IS AN EXAMPLE OF CONTINUOUS CULTURE?

For example, if a constant supply of cells is required, **a cell culture maintained in the log phase is** best; the conditions must therefore be continually monitored and adjusted accordingly so that the cells do not enter the stationary phase (see bacterial growth curve).

WHAT IS THE USE OF CONTINUOUS CULTURE?

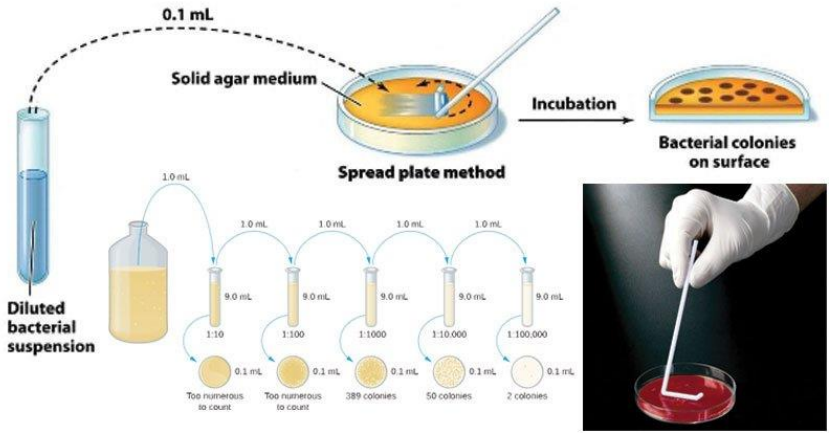
Continuous culture methods were developed to grow cells in a constant environment and have been used for decades to **study basic microbial physiology in a controlled and reproducible manner.**

METHODS OF ENUMERATION OF MICROORGANISMS

- The methods of enumeration in microbes can be divided into four categories.
- Direct methods involve counting the microbes, while indirect methods involve estimation.
- Viable methods only count cells that are metabolically active, while total counts include dead and inactive cells.

Direct/Viable

A direct/viable method involves a standard plate count, in which repeated dilutions of a sample are counted to calculate the count in the original sample.



SPREAD PLATE METHOD

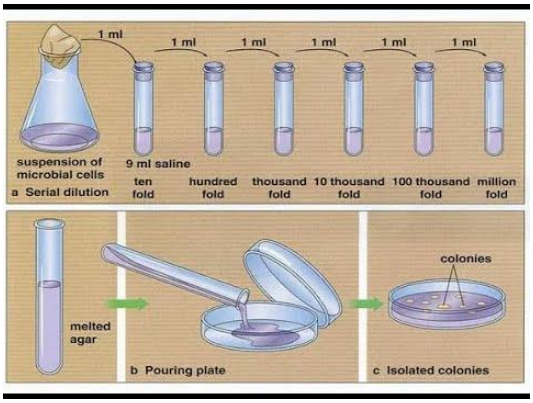
Indirect/Viable

Indirect/viable methods such as MPN (most probable number) involve making a statistical inference about the microbe count based on patterns of growth.

Direct/Total

The microbes are counted with the aid of fluorescent stains and dyes, which make the microbes visible with the aid of a fluorescent microscope.

POUR PLATE METHOD



Indirect/Total

Spectroscopy is a form of indirect/total enumeration, which involves estimating the amount of microbes based on the amount of light passed through the culture by a spectrophotometer.

PRESERVATION OF MICROORGANISMS

The methods are: 1. Agar Slant Cultures 2. Agar Slant Culture Covered with Oil (Parafin Method) 3. Saline Suspension 4. Preservation at Very Low Temperature 5. Preservation by Drying in Vacuum 6. Preservation by Freeze Drying (Byophilization).

Agar Slant Cultures:

- All microbiology laboratories preserve micro-organisms on agar slant.
- he slants are incubated for 24hr or more and are then stored in a refrigerator.
- These cultures are periodically transferred to fresh media.
- Time intervals at which the transfers are made which varies with the origin and condition of growth.

Agar Slant Culture Covered with Oil (Parafin Method):

- The agar slants are inoculated and incubated until good growth appears.
- They are then covered with sterile mineral oil to a depth of 1 cm above the tip of slant surface.
- Transfers are made by removing a loop full of the growth, touching the loop to the glass surface to drain off excess oil, inoculating a fresh medium and then preserving the initial stock culture.
- This is a simple and most economical method of preserving bacteria and fungi where they remain viable for several years at room temperature.
- The layer of paraffin prevents dehydration of the medium and by ensuring an aerobic condition, the microorganism remain in dormant state.

Saline Suspension:

Sodium chloride in high concentration is frequently an inhibitor of bacterial growth. Bacteria are suspended in 1% salt solution (sublethal concentration in screw cap tubes to prevent evaporation). The tubes are stored at room temperature. Whenever needed the transfer is made on agar slant.

Preservation at Very Low Temperature:

The organisms are suspended in nutrient broth containing 15% glycerol. The suspension is frozen and stored at -15°C to -30°C. The availability of liquid nitrogen (temp -196°C) provides another main preserving stock culture. In this procedure culture are frozen with a protective agent (glycerol or dimethane sulphoxide) in sealed ampoules. The frozen culture are kept in liquid nitrogen refrigerator.

Preservation by Drying in Vacuum:

The organisms are dried over calcium chloride in vacuum and are stored in the refrigerator.

Preservation by Freeze Drying (Byophilization):

- In this process the microbial suspension is placed in small vials. A thin film is frozen over the inside surface of the vial by rotating it in mixture of dry ice (solid carbon dioxide) and alcohol, or acetone at a temperature of −78ºC .
- The vials are immediately connected to a high vacuum line. This dries the organism while still frozen. Finally, the ampules are sealed off in a vacuum with small flame.
- These culture can be stored for several years at 40°C. This method is also employed for preservation of toxins, sera, enzymes and other biological material. To revive microbial cultures it is merely necessary to break open the vial aseptically, add a suitable stale medium, and after incubation make further transfers.
- The process permits the maintenance of longer number of culture without variation in characteristics of the culture and greatly reduces the danger of contamination.

MARUDHAR KESARI JAIN COLLEGE FOR WOMEN

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DEPARTMENT OF BIOTECHNOLOGY

MICROBIOLOGY

II B.Sc BIOTECHNOLOGY **SUBJECT CODE FBT31**

MS.S.ANUPRIYA

UNIT -4

CONTROL OF MICROBIAL GROWTH

- The control of microbial growth is necessary in many practical situations and significant advances in agriculture, medicine, and food science have been made through study of this area of microbiology. 'Control of microbial growth', as used here, means to inhibit or prevent growth of microorganisms.
- This control is affected in two basic ways: (1) by killing microorganisms or (2) by inhibiting the growth of microorganisms.
- Control of growth usually involves the use of physical or chemical agents which either kill or prevent the growth of microorganisms.
- Agents which kill cells are called cidal agents; agents which inhibit the growth of cells (without killing them) are referred to as static agents.
- Thus, the term bactericidal refers to killing bacteria, and bacteriostatic refers to inhibiting the growth of bacterial cells. A bactericide kills bacteria; a fungicide kills fungi, and so on.

PHYSICAL AGENTS TO CONTROL MICROORGANISMS

A. INTRODUCTION TO THE CONTROL OF MICROORGANISMS

Control of microorganisms is essential in order to prevent the transmission of diseases andinfection, stop decomposition and spoilage, and prevent unwanted microbial contamination.

Microorganisms are controlled by means of physical agents and chemical agents. Physical agents include such methods of control as high or low temperature, desiccation, osmotic pressure, radiation, and filtration. Control by chemical agents refers to the use of disinfectants, antiseptics, antibiotics, and chemotherapeutic antimicrobial chemicals.

Basic terms used in discussing the control of microorganisms include:

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

2. Disinfection
Disinfection is the elimination of microorganisms from inanimate objects or surfaces.

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

3. Disinfectant

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

4. Antiseptic
An antiseptic is an agent that kills or inhibits growth of microbes but is safe to use on humantissue.

6. Sanitizer
A sanitizer is an agent that reduces, but may not eliminate, microbial numbers to a safe level.

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

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

Keep in mind that when evaluating or choosing a method of controlling microorganisms, you must consider the following factors which may influence antimicrobial activity:

1. the concentration and kind of a chemical agent used;

2. the intensity and nature of a physical agent used;

3. the length of exposure to the agent;

4. the temperature at which the agent is used;

5. the number of microorganisms present;the organism itself; and

6. the nature of the material bearing the microorganism.

B. TEMPERATURE

Microorganisms have a minimum, an optimum, and a maximum temperature for growth. Temperatures **below the minimum** usually have a **static** action on microorganisms. They inhibitmicrobial growth by slowing down metabolism but do not necessarily kill the organism.
Temperatures **above the maximum** usually have a **cidal** action, since they denature microbialenzymes and other proteins. Temperature is a very common and effective way of controlling microorganisms.

1. High Temperature

Vegetative microorganisms can generally be killed at temperatures from 50°C to 70°C with moistheat. Bacterial **endospores**, however, are very resistant to heat and extended exposure to much higher temperature is necessary for their destruction. High temperature may be applied aseither moist heat or dry heat.

a. Moist heat

Moist heat is generally more effective than dry heat for killing microorganisms because of its ability to **penetrate** microbial cells. Moist heat kills microorganisms by **denaturing their proteins** (causes proteins and enzymes to lose their three-dimensional functional shape). It alsomay **melt lipids** in cytoplasmic membranes.

1. Autoclaving

Autoclaving employs **steam under pressure**. Water normally boils at 100°C; however, when putunder pressure, water boils at a higher temperature. During autoclaving, the materials to be sterilized are placed under **15 pounds per square inch of pressure** in a pressure-cooker type of apparatus. When placed under 15 pounds of pressure, the boiling point of water is raised to **121°C**, a temperature sufficient to kill bacterial endospores.

The time the material is left in the autoclave varies with the nature and amount of material beingsterilized. Given sufficient time (generally 15-45 minutes), autoclaving is **cidal** for both vegetative organisms and endospores, and is the most common method of sterilization for materials not damaged by heat.

2. Boiling water

Boiling water (100°C) will generally kill vegetative cells after about 10 minutes of exposure. However, certain viruses, such as the hepatitis viruses, may survive exposure to boiling waterfor up to 30 minutes, and endospores of certain *Clostridium* and *Bacillus* species may surviveeven hours of boiling.

b. Dry heat

Dry heat kills microorganisms through a process of **protein oxidation** rather than protein coagulation. Examples of dry heat include:

Hot air sterilization

Microbiological ovens employ very high dry temperatures: 171°C for 1 hour; 160°C for 2 hours or longer; or 121°C for 16 hours or longer depending on the volume. They are generally used only for sterilizing glassware, metal instruments, and other inert materials like oils and powders that are not damaged by excessive temperature.

1. Incineration

Incinerators are used to destroy disposable or expendable materials by burning. We alsosterilize our inoculating loops by incineration.

b.Pasteurization

Pasteurization is the mild heating of milk and other materials to kill **particular spoilage organisms or pathogens**. It does not, however, kill all organisms. Milk is usually pasteurized byheating to 71.6°C for at least 15 seconds in the flash method or 62.9°C for 30 minutes in the holding method.

2. **Low Temperature**

Low temperature **inhibits** microbial growth by **slowing down microbial metabolism**. Examplesinclude refrigeration and freezing. Refrigeration at 5°C slows the growth of microorganisms and keeps food fresh for a few days. Freezing at -10°C stops microbial growth, but generally does not kill microorganisms, and keeps food fresh for several months.

C. DESICCATION

Desiccation, or drying, generally has a **static** effect on microorganisms. Lack of water inhibits the action of microbial enzymes. Dehydrated and freeze-dried foods, for example, do not requirerefrigeration because the absence of water inhibits microbial growth.

D. OSMOTIC PRESSURE

Microorganisms, in their natural environments, are constantly faced with alterations in osmoticpressure. Water tends to flow through semipermeable membranes, such as the cytoplasmic membrane of microorganisms, towards the side with a higher concentration of dissolved materials (**solute**). In other words, **water moves from greater water (lower solute) concentration to lesser water (greater solute) concentration**.

When the concentration of dissolved materials or solute is higher inside the cell than it is outside,the cell is said to be in a **hypotonic environment** and water will flow **into the cell**. The rigid **cellwalls** of bacteria and fungi, however, **prevent bursting or plasmoptysis**. If the concentration ofsolute is the same both inside and outside the cell, the cell is said to be in an **isotonic environment**. Water flows equally in and out of the cell. Hypotonic and isotonic environments are not usually harmful to microorganisms. However, if the concentration of dissolved materials or solute is higher outside of the cell than inside, then the cell is in a **hypertonic environment**.
Under this condition, water flows **out of the cell**, resulting in shrinkage of the cytoplasmic membrane or **plasmolysis**. Under such conditions, the cell becomes **dehydrated** and its**growth is inhibited**.

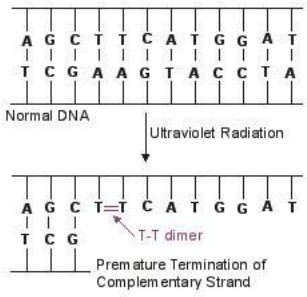
The canning of jams or preserves with a high sugar concentration inhibits bacterial growth through hypertonicity. The same effect is obtained by salt-curing meats or placing foods in a saltbrine. This **static action** of osmotic pressure thus prevents bacterial decomposition of the food. Molds, on the other hand, are more tolerant of hypertonicity. Foods, such as those mentioned above, tend to become overgrown with molds unless they are first sealed to exclude oxygen.(Molds are aerobic.)

E. RADIATION

1. Ultraviolet Radiation

The ultraviolet portion of the light spectrum includes all radiations with wavelengths from 100 nmto 400 nm. It has low wave-length and low energy. The microbicidal activity of ultraviolet (UV) light depends on **the length of exposure**: the longer the exposure the greater the cidal activity. It also depends on the **wavelength of UV used**. The most cidal wavelengths of UV light lie in the **260 nm - 270 nm range** where it is absorbed by nucleic acid.

In terms of its mode of action, UV light is absorbed by microbial DNA and causes adjacent thymine bases on the same DNA strand to covalently bond together, forming what are called**thymine-thymine dimers.**



As the DNA replicates, nucleotides do not complementary base pair with the thymine dimers and this terminates the replication ofthat DNA strand. However, **most of the damage from UV radiation actually comes from the cell trying to repair the damage to the DNA by a process called SOS repair**. In very heavily damaged DNA containing large numbers of thymine dimers, a process called SOS repair is activated as kind of a last ditch effort to repair the DNA. In this process, a gene product of the SOS system binds to DNA polymerase allowing it to synthesize new DNA across the damaged DNA. However, this

altered DNA polymerase loses its proofreading ability resulting in the synthesis of DNA that itself now contains many misincorporated bases. In other words, **UV radiation causes mutation**and can lead to faulty protein synthesis. With sufficient mutation, bacterial metabolism is blockedand the organism dies. Agents such as UV radiation that cause high rates of mutation are called **mutagens**.

The effect of this inproper base pairing may be reversed to some extent by exposing the bacteriato strong visible light immediately after exposure to the UV light. The visible light activates an enzyme that breaks the bond that joins the thymine bases, thus enabling correct complementarybase pairing to again take place. This process is called **photo reactivation**.

UV lights are frequently used to reduce the microbial populations in hospital operating rooms and sinks, aseptic filling rooms of pharmaceutical companies, in microbiological hoods, and inthe processing equipment used by the food and dairy industries.

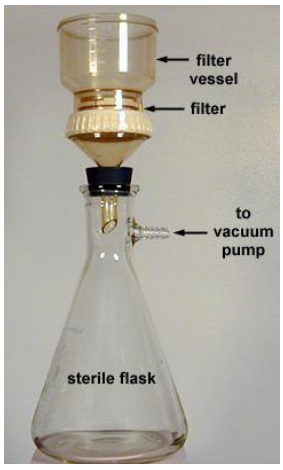
An important consideration when using UV light is that it has **very poor penetrating power**. Only microorganisms on the **surface** of a material that are exposed directly to the radiation are susceptible to destruction. UV light can also damage the eyes, cause burns, and cause mutationin cells of the skin.

2. Ionizing Radiation

Ionizing radiation, such as **X-rays and gamma rays**, has much more energy and penetrating power than ultraviolet radiation. It ionizes water and other molecules to form radicals (molecular fragments with unpaired electrons) that can **disrupt DNA molecules and proteins**. It is often used to sterilize pharmaceuticals and disposable medical supplies such as syringes, surgical gloves, catheters, sutures, and petri plates. It can also be used to retard spoilage in sea foods,meats, poultry, and fruits.

F. FILTRATION

Microbiological membrane filters provide a useful way of sterilizing materials such as vaccines, antibiotic solutions, animal sera, enzyme solutions, vitamin solutions, and other solutions that may be damaged or denatured by high temperatures or chemical agents. The filters contain pores small enough to prevent the passage of microbes but large enough to allow the organism-free fluid to pass through. The liquid is then collected in a sterile flask.



Filters with a pore diameter from 25 nm to 0.45 µm are usually used in this procedure. Filters can also be used to remove microorganisms from waterand air for microbiological testing.

BACTERIOLOGIC EXAMINATION OF WATER: COLIFORM COUNTS

The purpose of the bacteriological examination of water is to determine if there is a possibility of pathogens being present. Infectious diseases such as salmonellosis, typhoid fever, shigellosis, cholera, hepatitis A, amoebic dysentery, *Campylobacter* gastroenteritis, giardiasis, and other fecal-oral route diseases may be transmitted by fecally-contaminated water. The identification of pathogens, however, is quite difficult. Pathogens may not survive long in water and are usually present only in small quantity. Therefore, one usually tests for the presence of coliforms in water.

Coliforms are gram-negative, lactose-fermenting rods of the family Enterobacteriaceae. *Escherichia coli*, a fecal coliform, is normal flora of the intestines in humans and animals and is,therefore, a **direct indicator**

of fecal contamination of the water. The presence of coliforms would then indicate the possibility of fecal pathogens being present.

Two tests are frequently performed to monitor water: the fecal coliform count and the totalcoliform count.

1. The **fecal coliform count** tests specifically for the fecal coliform *E. coli*. M-FC medium is usedin this test and the plates are incubated at 45.5C. This temperature is **selective for fecal coliforms** (nonfecal coliforms will not grow at this temperature) which produce blue colonies. This test, however, requires a special water bath incubator to assure a temperature of 45.5C.

2. The **total coliform count** will detect **any coliforms (fecal and nonfecal)** present in the water.It is not as specific an indicator of fecal contamination, but is a useful **screening test**. M-coliform medium is used in this test and the plates are incubated at 37C. **Both fecal and nonfecal coliforms will grow and produce metallic green colonies**. Coliforms would indicate the **possibility of fecal contamination of the water**.

Both of these tests use the micropore membrane filter method. Different amounts of the water sample being tested are passed through a membrane filter. The water passes through and the bacteria are trapped on the surface of the filter. The filter is then placed in a petri plate on pads containing either M-FC or M-coliform medium. Colonies then form on the filter. By counting the number of colonies and knowing the volume of water sample used, the number of fecal coliformsor total coliforms per ml of water can be determined.

1. Describe how ionizing radiation kills microorganisms and state several common applications.

2. State the concept behind sterilizing solutions with micropore membrane filters.

3. State why filters are preferred over autoclaving for such materials as vaccines, antibioticsolutions, sera, and enzyme solutions.

DISINFECTANTS ANTISEPTICS AND SANITIZERS TO CONTROL MICROORGANISMS

A. DISINFECTANTS, ANTISEPTICS, AND SANITIZERS

Disinfection is the elimination of microorganisms from inanimate objects or surfaces, whereas **decontamination** is the treatment of an object or inanimate surface to make it safe to handle.

a. The term **disinfectant** is used for an agent used to disinfect inanimate objects or surfaces butis generally to toxic to use on human tissues.

b. The term **antiseptic** refers to an agent that kills or inhibits growth of microbes but is safe touse on human tissue.

c. The term **sanitizer** describes an agent that reduces, but may not eliminate, microbial numbersto a safe level.

Because disinfectants and antiseptics often work slowly on some viruses - such as the hepatitisviruses, bacteria with an **acid-fast cell wall** such as *Mycobacterium tuberculosis*, and especially bacterial **endospores**, produced by the genus *Bacillus* and the genus *Clostridium*, they are usually **unreliable for sterilization** - the destruction of **all** life forms.

There are a number of factors which influence the antimicrobial action of disinfectants andantiseptics,

including:

1. The **concentration** of the chemical agent.

2. The **temperature** at which the agent is being used. Generally, the lower the temperature, thelonger it takes to disinfect or decontaminate.

3. The **kinds of microorganisms** present. Endospore producers such as *Bacillus* species,*Clostridium* species, and acid-fast bacteria like *Mycobacterium tuberculosis* are harder to eliminate.

4. The **number of microorganisms** present. The more microorganisms present, the harder it isto disinfect or decontaminate.

5. The **nature of the material bearing the microorganisms**. Organic material such as dirt andexcreta interferes with some agents.

The best results are generally obtained when the initial **microbial numbers are low** and whenthe **surface to be disinfected is clean** and free of possible interfering substances.

There are 2 common antimicrobial **modes of action** for disinfectants, antiseptics, and sanitizers:

1. They may **damage the lipids and/or proteins of the semipermeable cytoplasmic membrane** of microorganisms resulting in **leakage of cellular materials** needed to sustain life.

2. They may **denature microbial enzymes and other proteins,** usually by disrupting the hydrogen and disulfide bonds that give the protein its three-dimensional functional shape. This**blocks metabolism**.

A large number of such chemical agents are in common use. Some of the more common groupsare listed below:

1. Phenol and phenol derivatives

Phenol (5-10%) was the first disinfectant commonly used. However, because of its toxicity and odor, phenol derivatives are now generally used. These include orthophenylphenol, hexachlorophene, triclosan, hexylresorcinol, and chlorhexidine. Orthophenylphenol is the agentin Lysol®, O-syl®, Staphene®, and Amphyl®. Hexachlorophene in a 3% solution is combined with detergent and is found in PhisoHex®. Triclosan is a chlorine-containing phenolic antisepticvery common in antimicrobial soaps and other products. Hexylresorcinol is in throat lozenges and ST-37. A 4% solution of chlorhexidine in isopropyl alcohol and combined with detergent (Hibiclens® and Hibitane®) is a common handwashing agent and surgical handscrub. These agents kill most bacteria, most fungi, and some viruses, but are usually ineffective against endospores. They **alter membrane permeability and denature proteins**.

2. Soaps and detergents

Soaps are only mildly microbicidal. Their use aids in the **mechanical removal** of microorganisms by breaking up the oily film on the skin (emulsification) and reducing the surfacetension of water so it spreads and penetrates more readily. Some cosmetic soaps contain addedantiseptics to increase antimicrobial activity.

Detergents may be anionic or cationic. **Anionic** (negatively charged) detergents, such as laundry powders, **mechanically remove microorganisms and other materials** but are not very microbicidal. **Cationic** (positively charged) detergents **alter membrane permeability and denature proteins**. They are effective against many vegetative bacteria, some fungi, and some viruses. However, bacterial endospores and certain

bacteria such as *Mycobacterium tuberculosis* and *Pseudomonas* species are usually resistant. They are also inactivated by soaps and organic materials like excreta. Cationic detergents include the quaternary ammonium compounds such as benzalkonium chloride, zephiran, diaprene, roccal, ceepryn, and phemerol.

3. Alcohols

70% solutions of ethyl or isopropyl alcohol are effective in killing vegetative bacteria, enveloped viruses, and fungi. However, they are usually ineffective against endospores and non-envelopedviruses. Once they evaporate, their cidal activity will cease. Alcohols **denature membranes** andare often combined with other disinfectants, such as iodine, mercurials, and cationic detergents for increased effectiveness.

4. Acids and alkalies

Acids and alkalies **alter membrane permeability and denature proteins and other molecules**.Salts of **organic acids**, such as calcium propionate, potassium sorbate, and methylparaben, are commonly used as food preservatives. Undecylenic acid (Desenex®) is used for dermatophyte infections of the skin. An example of an **alkali** is lye (sodium hydroxide).

5. Heavy metals

Heavy metals, such as mercury, silver, and copper, **denature proteins**. Mercury compounds (mercurochrome, metaphen, merthiolate) are only bacteriostatic and are not effective against endospores. Silver nitrate (1%) is sometimes put in the eyes of newborns to prevent gonococcalophthalmia. Copper sulfate is used to combat fungal diseases of plants and is also a common algicide. Selinium sulfide kills fungi and their spores.

6. Chlorine

Chlorine gas reacts with water to form **hypochlorite ions**, which in turn **denature microbial enzymes**. Chlorine is used in the chlorination of drinking water, swimming pools, and sewage.Sodium hypochlorite is the active agent in household bleach. Calcium hypochlorite, sodium hypochlorite, and chloramines (chlorine plus ammonia) are used to sanitize glassware, eating utensils, dairy and food processing equipment, hemodialysis systems, and treating water supplies.

7. Iodine and iodophores

Iodine also **denatures microbial proteins**. Iodine tincture contasns a 2% solution of iodine and sodium iodide in 70% alcohole. Aqueous iodine solutions containing 2% iodine and 2.4% sodiumiodide are commonly used as a topical antiseptic. **Iodophores** are a combination of iodine and an inert polymers such as polyvinylpyrrolidone that reduces surface tension and slowly releases the iodine. Iodophores are less irritating than iodine and do not stain. They are generally effective against vegetative bacteria, *Mycobacterium tuberculosis*, fungi, some viruses, and
some endospores. Examples include Wescodyne®, Ioprep®, Ioclide®, Betadine®, and Isodine®.

8. **Aldehydes.** Aldehydes, such as formaldehyde and glutaraldehyde, **denature microbial proteins**. Formalin (37% aqueous solution of formaldehyde gas) is extremely active and kills most forms of microbial life. It is used in embalming, preserving biological specimens, and in preparing vaccines. Alkaline glutaraldehyde (Cidex®), acid glutaraldehyde (Sonacide®), and glutaraldehyde phenate solutions (Sporocidin®) kill vegetative bacteria in 10-30 minutes and endospores in about 4 hours. A 10 hour exposure to a **2% glutaraldehyde** solution can be usedfor **cold sterilization** of materials.

9. Ethylene oxide gas

Ethylene oxide is one of the very few chemicals that can be relied upon for **sterilization** (after4-12 hours exposure). Since it is explosive, it is usually mixed with inert gases such as freon orcarbon dioxide. **Gaseous chemosterilizers**, using ethylene oxide, are commonly used to sterilize heat-sensitive items such as plastic syringes, petri plates, textiles, sutures, artificial heart valves, heart-lung machines, and mattresses. Ethylene oxide has very high penetrating power and **denatures microbial proteins**. Vapors are toxic to the skin, eyes, and mucous membranes and are also carcinogenic. Another gas that is used as a sterilant is **chlorine dioxide** which denatures proteins in vegetative bacteria, bacterial endospores, viruses, and fungi.

B. EVALUATION OF DISINFECTANTS, ANTISEPTICS, AND SANITIZERS

It is possible to evaluate disinfectants, antiseptics, and sanitizers using either in vitro or in vivotests. An ***in vitro*** test is one done under **artificial, controlled laboratory conditions**. An ***in vivo*** test is one done under the **actual conditions of normal use**.

A common *in vitro* test is to compare the antimicrobial activity of the agent being tested with that of phenol. The resulting value is called a phenol coefficient and has some value in comparing the strength of disinfectants under standard conditions. Phenol coefficients may be misleading, however, because as mentioned earlier, the killing rate varies greatly with the conditions under which the chemical agents are used. The concentration of the agent, the temperature at which it is being used, the length of exposure to the agent, the number and kinds of microorganisms present, and the nature of the material bearing the microorganisms all influence the antimicrobialactivity of a disinfectant. If a disinfectant is being evaluated for possible use in a given *in vivo* situation, it must be **evaluated under the same conditions in which it will actually be used**.

A. EFFECTIVENESS OF HAND WASHING

There are 2 categories of microorganisms, or flora, normally found on the hands. **Resident flora**are the normal flora of the skin. **Transient flora** are the microorganisms you pick up from what you have been handling. It is routine practice to wash the hands prior to and after examining a patient and to do a complete regimented surgical scrub prior to going into the operating room.
This is done in order to remove the potentially harmful transient flora, reduce the number ofresident flora, and disinfect the skin.

Actual sterilization of the hands is not possible since microorganisms live not only on the surfaceof the skin but also in deeper skin layers, in ducts of sweat glands, and around hair follicles.
These normal flora are mainly nonpathogenic staphylococci and diphtheroid bacilli.

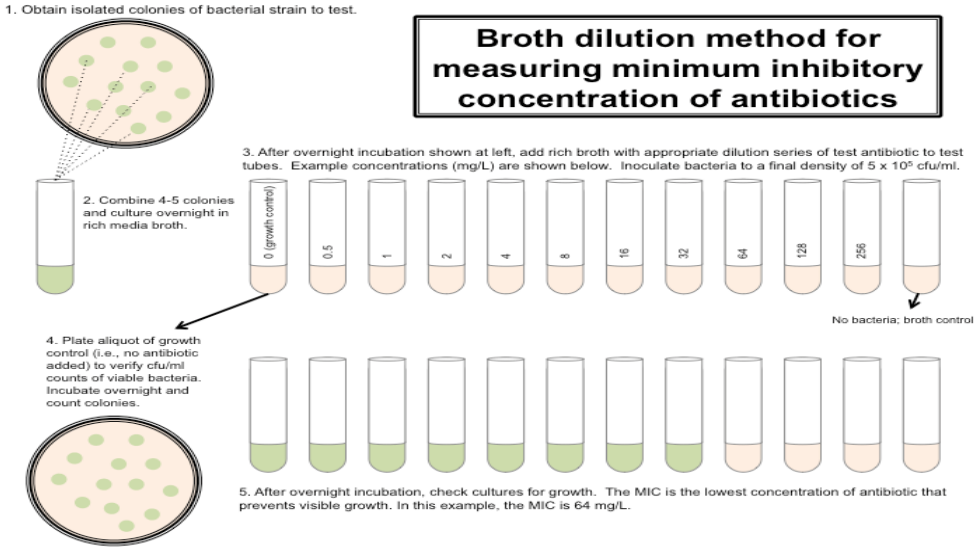
ANTIMICROBIAL CHEMOTHERAPY EVALUATION

TUBE DILUTION

- The tube dilution test is the standard method for determining levels of resistance to an antibiotic. Serial dilutions of the antibiotic are made in a liquid medium which is inoculated with a standardized number of organisms and incubated for a prescribed time. The lowest concentration (highest dilution) of antibiotic preventing appearance of turbidity is considered to be the minimal inhibitory concentration (MIC). At this dilution the antibiotic is bacteriostatic.
- Additionally, the minimal bactericidal concentration (MBC) can be determined by sub culturing the contents of the tubes onto antibiotic-free solid medium and examining for bacterial growth.
- Although the tube dilution test is fairly precise, the test is laborious because serial dilutions of the antibiotic must be made and only one isolate can be tested in each series of dilutions.

PROCEDURE

- Number sterile capped test tubes 1 through 9. All of the following steps are carried out using aseptic technique.
- Add 2.0 ml of tetracycline solution (100 ug/ml) to the first tube. 3. Add 1.0 ml of sterile broth to all other tubes.
- Transfer 1.0 ml from the first tube to the second tube.
- Using a separate pipette, mix the contents of this tube and transfer 1.0 ml to the third tube.
- Continue dilutions in this manner to tube number 8, being certain to change pipettes between tubes to prevent carryover of antibiotic on the external surface of the pipette.
- Remove 1.0 ml from tube 8 and discard it. The ninth tube, which serves as a control, receives no tetracycline.
- Suspend to an appropriate turbidity several colonies of the culture to be tested in 5.0 ml of Mueller-Hinton broth to give a slightly turbid suspension.
- Dilute this suspension by aseptically pipetting 0.2 ml of the suspension into 40 ml of Mueller-Hinton broth.
- Add 1.0 ml of the diluted culture suspension to each of the tubes. The final concentration of tetracycline is now one-half of the original concentration in each tube.
- Incubate all tubes at 35°C overnight.
- Examine tubes for visible signs of bacterial growth. The highest dilution without growth is the minimal inhibitory concentration (MIC).

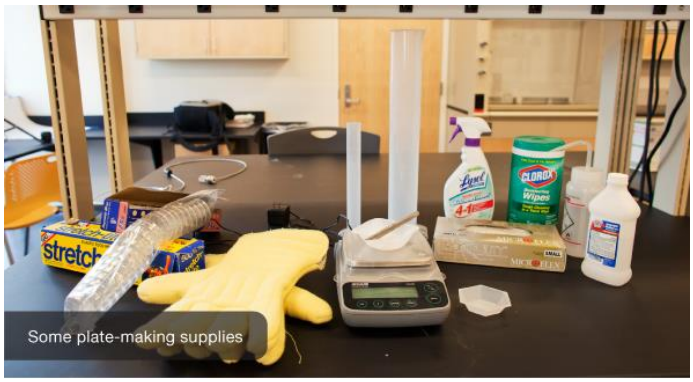


AGAR PLATE TECHNIQUE

- Agar plates are the standard solid support material for growing microorganisms. Microbial growth media contains nutrients and an energy source to fuel the microbes as they grow, and agar to keep the media in a semi-solid, gel-like state.
- On solid media, a single microbe will grow and divide to produce a "colony," a spot of identical descendants. Different types of microbes produce colonies with different characteristics-shape, color, texture-which help microbiologists determine if a culture is pure, or identify the types of microbes in a mixed sample.
- A number of biological supply companies sell pre-made plates, but making your own is much less expensive. With a little practice, you will find that it is very easy to make your own plates, and you will have the added flexibility of being able to customize recipes to suit your needs.

CHOOSE A RECIPE

- Choose a recipe from the Media Recipes page or use one of your own.
- Decide how many plates you will need. Our recipes will make 1 L (1000 mL) of media, enough to fill approximately forty 100 mm plates, but they can be scaled up or down as needed. Plan on using about 25 mL per 100 mm plate.



GATHER SUPPLIERS

- Media Recipe ingredients (see the Media Recipes page)
- sterile, polystyrene Petri dishes. 100 x 15 mm is the most common size, but 60 and 35 mm sizes also work
- glass container that will hold at least twice the volume of your media
- aluminum foil for covering your media container, or plastic wrap if you use a microwave
- autoclave, pressure cooker, microwave, or hot plate for sterilizing your media (see the Sterilizing Liquids page)
- heat-resistant gloves, hothands, or potholders for handling hot containers
- 70% ethyl or isopropyl (rubbing) alcohol
- household cleaner, 10% bleach, or disinfectant wipes for cleaning your work area
- graduated cylinder for measuring water
- balance for weighing solid ingredients
- tools for handling solid ingredients (such as weigh boats and scoopulas, or paper plates and spoons)

PREPARE MEDIA

Use a glass container (ideally an Erlenmeyer flask) that will hold at least twice the volume of your media.

1. Assemble the ingredients according to the recipe of your choice.
2. Cover with aluminum foil, or plastic wrap if you use a microwave.

STERILIZE

- Sterilize using one of the methods described on the Sterilizing Liquids page.
- One advantage of high-salt media is that typical contaminating microbes won't grow on it, so media with a salt concentration of at least 10% can be sterilized by boiling.
- **Make sure the agar dissolves completely.** In media with 15% or more salt, the agar may be slow to dissolve. The media may look cloudy, or you may see small, translucent lens-like objects floating in it. Continue boiling until the media is completely clear; this may take longer than 15 minutes. Incompletely dissolved agar will leave your media squishy or fragile.

Pour into plates

1. Prepare a suitable work area. Label the plates with the type of media you will pour into them.
2. Swirl the hot media vigorously to mix.
3. Cool the media until it is just cool enough to handle, about 20-30 minutes. You should be able to hold your hand again the container reasonably comfortably for a few seconds. If the media is too cool, it will start to solidify in the container. If it is too hot, it will leave excess condensation on the lids.
4. Swirl the media again to mix just before pouring; be careful not to incorporate bubbles.
5. Pour into plate until it covers the bottom, approximately 25 mL (see video below).
6. After several hours to overnight, return the plates to the plastic sleeve they came in or place them in a plastic bag. Label the bag with the media type and the date, and store upside down in a refrigerator.

   Plates will keep refrigerated for 4-6 week

MARUDHAR KESARI JAIN COLLEGE FOR WOMEN

VANIYAMBADI

DEPARTMENT OF BIOTECHNOLOGY

MICROBIOLOGY

II B.Sc BIOTECHNOLOGY **SUBJECT CODE FBT31**

MS.S.ANUPRIYA

UNIT -5

MICROBES AS A SOURCE OF PROTEIN

SINGLE CELL PROTEINS

INTRODUCTION

- Single cell protein (SCP) refers to dead, dry cells of microorganisms, such as yeast, fungi, bacteria and algae
- These microorganisms grow on various carbon sources for their protein content
- The term, 'single cell protein' was firstly used by Carol Wilson in 1967 by replacing the less aesthetic terminology, 'petro protein' , 'microbial protein'
- The majority of microorganisms used are unicellular, the protein content from them is called , 'single cell protein'
- Because of its superior nutritional quality in terms of protein content and a very good supplement for animal feed, yeast is mostly chosen for SCP
- SCP do contain contents, such as vitamins, fats and minerals etc.
- Protein shortage in the Third World nations prompted in the cultivation of SCP on large scale which resulted in the development of SCP technology for livestock and human consumption
- It is estimated that SCP fermenters covering one third of a square mile can provide 10% of the World's protein requirement
- Need for microbial production of scp
- It is an idea to solve global food scarcity
- SCP can give relief to the agriculture sector which uses large area for production of protein crops
- Production per unit area in the agriculture sector is low,
- 10% of the World's protein requirement can be met by SCP technology by using only one third of a square mile for SCP production
- Climatic factor effects agriculture, whereas SCP technology is not affected by climate
- Scenario is also not so encouraging for animal protein too
- SCP has many advantages, such as

- High protein content
- Contains all the essential amino acids

- Some microorganisms are highly rich in vitamins
- High ratio of surface area to volume
- Small doubling time
- High growth rate
- Flexibility in the use of substrate
- Independence of cultivable land and climate
- Works on continuous basis
- Eco-friendly
- Cost effective
- Energy efficient
- Can also be genetically controlled

sources of microorganisms and the selection criteria

Microorganisms, such as algae, fungi, protozoa and bacteria are used for production of SCP

2 the basis for their selection are:

- Ability to utilize carbon and nitrogen sources
- Moderate growth conditions
- Tolerance to ph, temperature, and mineral concentrations
- Resistance against viral infection
- Non-toxicity
- Non-pathogenicity
- Acceptable nutritive value of cell mass
- Among algae, *Spirulina* is used most extensively
- Biomass from *Chlorella*, *Senedesmus* and *Dunalliella* used on large scale
- Main problems for SCP from algae are their foul odor and tastelessness
- Fungi species, such as *Spergillus*, *Fusarium*, *Candida*, *Chaetomium*, *Trihoderma*, *Penicillium* etc are good candidates for SCP production, due to:

wide range of substrate utilization

ability to withstand abiotic conditions

Bacteria, such as *Bacillus*, *Lactobacillus*, *Pseudomonas*, *Aesonomas* are used for SCP but the success is not so encouraging

Mixed cultures have shown better results with respect to stability and resistance to contamination

- ❖ Substrates suitable for SCP production
- ❖ Carbon dioxide (CO2) and sunlight are the main source for growth of algae
- ❖ Fungal species are grown on various substrates, such as
- ❖ Sulfite waste liquor
- ❖ Prawn shell wastes
- ❖ Dairy waste, whey
- ❖ Molasses, etc.

A list of the microorganisms used for the production of Single Cell Protein is as follows:

Fungi

- Aspergillus fumigatus
- Aspergillus niger
- Rhizopus cyclopean

Yeast

- Saccharomyces cerevisiae
- Candida tropicalis
- Candida utilis

Algae

- Spirulina (spa)
- Chlorella pyrenoidosa
- Chondrus crispus

Bacteria

- Pseudomonas fluorescens
- Lactobacillus
- Bacillus megaterium

USES (APPLICATIONS) OF SCP

- ❖ Provides instant energy
- ❖ It is extremely good for healthy eyes and skin
- ❖ Provides the best protein supplemented food for undernourished children
- ❖ Serves as a good source of vitamins, amino acids, minerals, etc.

USED IN THERAPEUTIC AND NATURAL MEDICINES FOR

- ❖ Controlling obesity
- ❖ Lowers blood sugar level in diabetic patients
- ❖ Reducing body weight, cholesterol and stress
- ❖ Prevents accumulation of cholesterol in the body.

USED IN COSMETICS PRODUCTS FOR

- ❖ Maintaining healthy hair
- ❖ Production of different herbal beauty products, like- biolipstics, herbal face cream, etc.

USED IN POULTRY AND DAIRY FARMS

- ❖ As it serves as an excellent and convenient source of proteins and other nutrients, it is
- ❖ Widel used for feeding cattle, birds, fishes, etc.

BASIC STEP INVOLVED IN SCP PRODUCTION

- Provision of carbon source which may need physical and/or chemical pretreatment.
- Perry cellulose – Pretreated chemically by acid hydrolysis or enzymatically by using cellulase enzyme.
- Lignocellulose- alkali or acid hydrolysis or steam exposure.
- Addition of nitrogen, phosphorus and other nutrients to the carbon sources needed to support optimal growth of the selected micro-organisms.
- Prevention of contamination by maintaining sterile or hygienic condition.
- The medium component may be heated or sterilized by filtration and fermentation equipment are also sterilized.
- Inoculate the selected microorganisms in a pure state.

Fermentation:

- The fermentation process requires a pure culture of chosen organism that is in the correct physiological state.
- A production fermenter is used which for multiplication of organism and drawing the culture medium in the steady state and then cell separation is done.

Harvesting or product recovery:

- Different methods are used for product recovery depending upon type of micro-organisms used for SCP production.

Algal mass:

- Recovered by concentration, de watering and drying.
- Sometimes, flocculant (E.g. Aluminium sulphate and calcium hydroxide can also be used.
- For *Spirullina,* it can float on surface water. So, it can be filtered and suspension is dried with hot air to get fine powder.

Bacterial biomass:

- Many problems related with recovery of bacterial cell like they are very small and have cell density in the order of 10-20 gm/litre.
- Centrifugation cost is also high.
- A device has been developed for separation of *Methylomonas clara* from methanol containing culture medium which is based on coagulation and centrifugation.

Yeast biomass:

- Yeast cells are small in size and can be recovered by decantation, centrifugation, by washing and dried treatment method.

Fungal biomass: mycoprotein

- 1-2% biomass growing in a fermenter

Purification of SCP processing for food:

- Liberation of cell proteins by destruction of ingestible cell walls.
- This can be done by chemical treatment by using acid, base, detergents, enzyme treatment, heating, freezing and thawing.
- Removal of nucleic acid, Degradation of nucleic acid produces uric acid which may accumulate to damaging level in human because human beings donot possess uricase activity.
- It is therefore necessary to reduce nucleic acids to acceptable low level especially in SCP intended for human use.

It can be reduced by activation of endogenous rnase by a brief heat treatment. E.g. 20 mins at 64°c reduces RNA from 10% to 1% of dry weight in case of *Fusarium graminerium.*

–alkalinehydrolysis

–chemicalextraction

– suitable manipulation of growth and physiology of microbial cell.

ROLE OF MICROBES IN FOOD SPOILAGE

FOOD SPOILAGE

- **Food spoilage is a metabolic process that causes foods to be undesirable or unacceptable for human consumption due to changes in sensory characteristics.**
- Spoilage of food is identified by off-colors, off-odors, softening of vegetables, fruits, and slime production.
- Spoilage may arise from insect damage, physical damage, and indigenous enzyme activity in food or by microorganisms (bacteria, viruses, fungi).
- The spoilage microbe's common inhabitants are **soil**, water, or the intestinal tracts of animals or they are dispersed through the air and water.
- Food spoilage by microorganisms depends upon intrinsic (pH, water activity, nutrient content, oxidation-reduction potential, antimicrobial property) and extrinsic factors (temperature, relative humidity, pressure).
- Different spoilage-causing microorganisms have different nutrients requirements.
- Microorganisms are the biological agents that cause foodborne diseases when consumed however the microorganisms not only cause spoilage, some of them are beneficial for food fermentation.

BACTERIA IN FOOD

- Food is most commonly spoiled by bacteria as it can grow in a wide variety of conditions however bacteria are used for beneficial fermentations of pickles, milk products, and some fermented vegetable products.
- Bacteria do not grow at a water activity level below 0.91 and require neutral pH (6.5-7) to cause food spoilage (e.g. milk, meat, green vegetables, fruits, etc.)
- Some bacteria are capable of spore formation so they are highly heat resistant and some are capable of producing heat-resistant toxins.
- The consumption of such spoiled food leads to foodborne illness.
- The most common bacteria that cause food spoilage are-
    - Gram-positive bacteria such as *Staphylococcus aureus, Bacillus* spp*, Clostridium* spp, Lactic acid bacteria (LAB), *Leuconostoc* spp, *Streptococcus* spp, *Brochothrix* spp, *Weissella* spp, *Mycobacterium bovis,* etc.
    - Gram-negative bacteria such as *Salmonella* spp*, Shigella, Vibrio* spp, *Escherichia coli, Campylobacter jejuni*, *Yersinia enterocolitis, Brucella* spp, *Coxiella burnetii, Aeromonas* spp, *Plesiomonas shigelloides,* etc.
- These bacteria cause off-odors and off-flavors, discolorations, gas production, slime production, and decreases in pH in food.

FUNGI IN FOOD

- Fungi is the most abundant group of microorganisms that plays important role in food spoilage.
- Fungi are osmotrophic they obtain their nutrients by absorption.
- Fungi can be divided into mold and yeast.

Molds

- Molds are the most common food spoilage-causing microorganisms.
- Molds grow on the surface of food (they require free oxygen for growth) and in a wide range of pH values (from 2 to 8.5), but the majority of them prefer acidic pH.
- Molds can grow at very low water activity levels (0.7–0.8) on dried foods (e.g. grains, beans, peanuts, and some spices)
- The most common food spoilage causing molds are *Mucor, Aspergillus* spp*, Rhizopus* spp*, Penicillium* spp*, Alternaria* spp*, Bothrytis, Byssochlamys, Fusarium* spp.
- This mold causes off-flavors, mycotoxins contamination, discoloration, rotting, and is externally visible in the food.

Yeasts

- Compared to bacteria and molds, yeasts play a minor role in food spoilage
- Yeasts can grow with or without oxygen and are used for beneficial fermentation in bread and alcoholic drinks fermentation.
- They often spoil food that has high sugar or salt content (e.g. maple syrup, pickles, jams, soy sauce, and sauerkraut.)
- Yeasts require a water activity level of 0.90–0.95 for growth and they can grow in a wide range of pH (3 – 8) but in general, they prefer acidic pH (4.5-5.5).
- Most commonly food spoilage causing yeasts are *Zygosaccharomyces* spp, *Saccharomyces* spp., *Candida* spp, *Dekkera* spp
- These yeasts cause a change in color, a change in texture, an unpleasant odor, or an undesirable taste in food.

PROTOZOA IN FOOD

- Protozoa are one-celled microorganisms without a rigid cell wall and the transmissible form of these organisms is termed cysts.
- Protozoan parasites are highly associated with foodborne and water-borne outbreaks of disease. The water and food act as a carrier for transmission of protozoan parasites from one host to another.
- The most common foodborne parasites are *Giardia lamblia*, *Entamoeba histolytica*, *Cyclospora cayetanensis, Toxoplasma gondii,* and *Trichinella spiralis.*

ALGAE IN FOOD

- Algae are primary producers which are a source of different nutrients and they are usually of aquatic habitats.
- They contaminate the water source with their toxin and cause them to accumulate in fish and marine life. The toxic may or may not be harmful to marine lives. When such fish or other marine lives are consumed by humans, it leads to foodborne illness.

- The algae that cause poisoning are *Gonyaulax catenella, Gonyaulax tamarensis, Gambierdiscus toxicus, Ptychodiscus brevis, Microcystis aeruginosa*, Blue-green Algae.

VIRUSES IN FOOD

- Viruses are obligate intracellular parasites that cause a wide range of diseases in plants, animals, and humans.
- Viruses need specific living cells to replicate and therefore they cannot replicate in food or water. The water and food act as a carrier for transmission of viruses from one host to another.
- Foodborne viruses are quite stable outside the host and are acid-resistant.
- Some of the foodborne viruses are Norovirus, *Hepatitis A virus* (HAV), *Hepatitis E virus* (HEV), *Astrovirus* (AstV), *Rotavirus* (RV), *Coronavirus*, *Sapovirus* (SaV).

PRIONS IN FOOD

- Prions are infectious disease-causing agents which are the normal protein of a brain that gets misfolded that lacks genome resulting in a pathological, infectious conformation.
- Once misfolded, it can induce other normally folded proteins to become misfolded.
- Prion diseases can affect both humans and animals. It can also get transferred from animal to human through the consumption of infected meat and meat products.
- Some examples of prion disease are Bovine spongiform encephalopathies' (BSE), Scrapie, Chronic wasting disease (CWD), Creutzfeldt-Jacob Disease (CJD).

ROLE OF MICROBES IN HUMAN DISEASES

INTRODUCTION

A few harmful microbes, for example less than 1% of bacteria, can invade our body (the host) and make us ill. Microbes cause infectious diseases such as flu and measles.

There is also strong evidence that microbes may contribute to many non–infectious chronic diseases such as some forms of cancer and coronary heart disease. Different diseases are caused by different types of micro-organisms. Microbes that cause disease are called pathogens.

Infectious disease	Microbe that causes the disease	Type of microbe
Cold	Rhinovirus	Virus
Chickenpox	*Varicella zoster*	Virus

German measles	Rubella	Virus
Whooping cough	*Bordatella pertussis*	Bacterium
Bubonic plague	*Yersinia pestis*	Bacterium
TB (Tuberculosis)	*Mycobacterium tuberculosis*	Bacterium
Malaria	*Plasmodium falciparum*	Protozoan
Ringworm	*Trichophyton rubrum*	Fungus
Athletes' foot	*Trichophyton mentagrophytes*	Fungus

It is important to remember that:

- A pathogen is a micro-organism that has the potential to cause disease.
- An infection is the invasion and multiplication of pathogenic microbes in an individual or population.
- Disease is when the infection causes damage to the individual's vital functions or systems.
- An infection does not always result in disease!

To cause an infection, microbes must enter our bodies. The site at which they enter is known as the portal of entry.

Microbes can enter the body through the four sites listed below:

- Respiratory tract (mouth and nose) e.g. influenza virus which causes the flu.
- Gastrointestinal tract (mouth oral cavity) e.g. *Vibrio cholerae* which causes cholera.
- Urogenital tract e.g. *Escherichia coli* which causes cystitis.
- Breaks in the skin surface e.g. *Clostridium tetani* which causes tetanus.

To make us ill microbes have to:

- reach their target site in the body;
- attach to the target site they are trying to infect so that they are not dislodged;
- multiply rapidly;

- obtain their nutrients from the host;
- avoid and survive attack by the host's immune system.

HEPATITIS

- **Hepatitis**, inflammation of the liver that results from a variety of causes, both infectious and non infectious.
- Infectious agents that cause hepatitis include viruses and parasites. Non infectious causes include certain drugs and toxic agents.
- In some instances hepatitis results from an auto immune reaction directed against the liver cells of the body.

SIGNS AND SYMPTOMS

- The signs and symptoms of acute viral hepatitis result from damage to the liver and are similar regardless of the hepatitis virus responsible.
- Patients may experience a flulike illness, and general symptoms include nausea, vomiting, abdominal pain, fever, fatigue, loss of appetite, and, less commonly, rash and joint pain. Sometimes jaundice, a yellowing of the skin and eyes, will develop.
- The acute symptomatic phase of viral hepatitis usually lasts from a few days to several weeks; the period of jaundice that may follow can persist from one to three weeks.
- Complications of acute viral hepatitis include fulminant hepatitis, which is a very severe, rapidly developing form of the disease that results in severe liver failure, impaired kidney function, difficulty in the clotting of blood, and marked changes in neurological function. Such patients rapidly become comatose; mortality is as high as 90 percent.
- Another complication is chronic hepatitis, which is characterized by liver cell death and inflammation over a period greater than six months.

VIRAL CAUSES

- Most cases of hepatitis are caused by viral infection.
- The viruses that give rise to liver inflammation include cytomegalovirus; yellow-fever virus; Epstein-Barr virus; herpes simplex viruses; measles, mumps, and chickenpox viruses; and a number of hepatitis viruses. The term *viral hepatitis*, however, usually is applied only to those cases of liver disease caused by the hepatitis viruses.
- There are seven known hepatitis viruses, which are labelled A, B, C, D, E, F, and G. Hepatitis A, E, and F viruses are transmitted through the ingestion of contaminated food or water (called the fecal-oral route); the spread of these agents is aggravated by crowded conditions and poor sanitation. The B, C, D, and G viruses are transmitted mainly by blood or bodily fluids; sexual contact or exposure to contaminated blood are common modes of transmission.

HOW DO MICROBES MAKE PEOPLE SICK WITH HEPATITIS?

- Otherwise known as germs, microbes are microscopic organisms, such as a bacteria, fungi, viruses or protozoa that are so small you need to use a microscope and

special staining techniques to see them. The word *microbe* is often more convenient to use than the word *microorganism*, but the two terms generally mean the same thing. In many situations, though, *microbe* refers to just the harmful microorganisms (the ones that cause disease), whereas *microorganisms* refer to all microscopic life.

- ❖ Microbes are abundant in all life on earth and live everywhere, including in the air we breathe, soil, water, plants, animals and in the human body. While some microbes are beneficial to health, others are disease-causing.

What Do Microbes Do?

- ❖ Most of the microbes in the human body are either beneficial or harmless. The beneficial ones help keep us healthy and perform the basic activities of life, such as digesting our food, absorbing nutrients, and producing vitamins and anti-inflammatory proteins. The human body is first populated with these healthy microbes during birth when it passes through a woman's vaginal canal.
- ❖ However, there are more dangerous microbes that exist in the human body as well. For example, about one-third of people harbor *Staphylococcus aureus* in their nasal passages. This bacterium is usually benign but can turn dangerous when it beats out competition from healthy microbes that normally keep it in check. that can turn virulent. Of particular concern today is the increased number of disease-causing microbes that have developed resistance to antibiotics and other treatments.

MICROBES AS THE CAUSE OF HEPATITIS

Hepatitis is an inflammation of the liver, and this can be caused by toxic chemicals, certain medications, and, most often, infection with a range of microbes. There are five known types of viral hepatitis, commonly known as hepatitis A, B, C, D, and E. Each of these five viruses can lead to short-term (acute) or long-term (chronic) infection, which can result in liver scarring, failure or cancer.

Because the five viruses causing hepatitis are different, they are transmitted differently as well:

- Hepatitis A and E are spread through ingestion of food or water that has been contaminated by fecal material from an infected person, also known as the fecal-oral route of transmission.
- Hepatitis B is spread through contact with infected blood or other bodily fluids such as saliva or semen.
- Hepatitis C is transmitted through exposure to infected blood.
- Hepatitis D is also transmitted through contact with infected blood, but only people already infected with hepatitis B are at risk because hepatitis B allows hepatitis D to survive in the body.

Treatments for hepatitis focus on suppressing the virus in the human body and thereby protecting the liver and other organs from damage.

HOW TO PREVENT EXPOSURE TO HEPATITIS-CAUSING MICROBES

Effective vaccines are available to help protect against hepatitis A and B. The best way to protect yourself against exposure to the other hepatitis viruses is by:

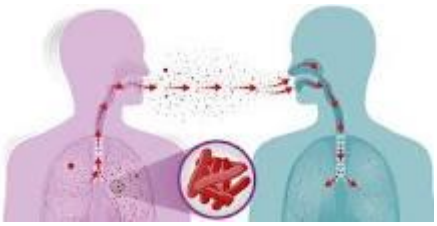
- Using condoms
- Avoiding sharing needles, toothbrushes and razors
- Demanding a sterile environment and safe practice during health procedures, or when getting tattoos and piercings
- Washing hands thoroughly after using the restroom
- Being careful when eating raw food and drinking bottled water when traveling if you are unsure of sanitation

TUBERCULOSIS

- A person may develop TB after inhaling *Mycobacterium tuberculosis* (*M. tuberculosis*) bacteria.

- When TB affects the lungs, the disease is the most contagious, but a person will usually only become sick after close contact with someone who has this type of TB.

HOW DOES TB BACTERIA WORK?



When a person breathes in TB bacteria, the **bacteria can settle in the lungs and begin to grow**. From there, they can move through the blood to other parts of the body, such as the kidney, spine, and brain. TB disease in the lungs or throat can be infectious. This means that the bacteria can be spread to other people.

WHAT MICROBES CAUSE TUBERCULOSIS?

Tuberculosis (TB) is caused by a type of bacterium called **Mycobacterium tuberculosis**. It's spread when a person with active TB disease in their lungs coughs or sneezes and someone else inhales the expelled droplets, which contain TB bacteria.
What is the role of tuberculosis?

When a person gets active TB disease, it means TB bacteria are **multiplying and attacking the lung(s) or other parts of the body**, such as the lymph nodes, bones, kidney, brain, spine and even the skin. From the lungs, TB bacteria move through the blood or lymphatic system to different parts of the body.

TAXONOMY

Order – Actinomycetales

Family – Mycobacteriacea

Genus – Mycobacterium

Over 130 know described species

Most are non-pathogenic (soil/water organisms)

Usually grouped in 2 divisions

Typical Mycobacteria (MTb complex)

Atypical Mycobacteria

MOTT – slow growers other than tuberculosis

Rapid growers

GENERAL CHARACTERISTICS OF MYCOBACTERIUM

Gram Positive (wont actually stain)

Slightly curved rod-shaped bacilli, aerobic, non-motile

Can show filimentous branching like fungus "myco"

Thick lipid rich cell wall

Can remain dormant, non spore forming

Multiplies slowly (18-24 hour generation time)

Acid Fast – resists stain decolorization with acid/alcohol

SYMPTOMS

Latent TB: A person with latent TB will have no symptoms, and no damage will show on a chest X-ray. However, a blood test or skin prick test will indicate that they have TB infection.

Active TB: A person with TB disease may experience a cough that produces phlegm, fatigue, a fever, chills, and a loss of appetite and weight. Symptoms typically worsen over time, but they can also spontaneously go away and return.

BEYOND THE LUNGS

TB usually affects the lungs, though symptoms can develop in other parts of the body. This is more common in people with weakened immune systems.

TB can cause:

- persistently swollen lymph nodes, or "swollen glands"
- abdominal pain
- joint or bone pain
- confusion
- a persistent headache

CAUSES

- *M. tuberculosis* bacteria cause TB. They can spread through the air in droplets when a person with pulmonary TB coughs, sneezes, spits, laughs, or talks.
- Only people with active TB can transmit the infection. However, most people with the disease can no longer transmit the bacteria after they have received appropriate treatment for at least 2 weeks.

HOW DOES TUBERCULOSIS BACTERIA AFFECT THE BODY?

- When a person gets active TB disease, it means TB bacteria are **multiplying and attacking the lung(s) or other** parts of the body, such as the lymph nodes, bones, kidney, brain, spine and even the skin. From the lungs, TB bacteria move through the blood or lymphatic system to different parts of the body.

WHAT IS TYPHOID FEVER?

- Typhoid fever and paratyphoid fever are similar diseases caused by bacteria. *Salmonella* Typhi bacteria cause typhoid fever. *Salmonella* Paratyphi bacteria cause paratyphoid fever.
- People infected with these bacteria can spread them to others. This typically happens when an infected person uses the bathroom and does not wash their hands. The bacteria can stay on their hands and contaminate everything that the person touches, including any food and drinks.
- In countries with poor sanitation, the water used to rinse and prepare food and beverages can also be contaminated with these bacteria. Travelers who eat foods or drink beverages contaminated with these bacteria can then get sick.
- Typhoid fever and paratyphoid fever cause similar symptoms. People with these diseases usually have a fever that can be as high as 103–104°F (39–40°C). They also may have weakness, stomach pain, headache, diarrhea or constipation, cough, and loss of appetite. Some people have a rash of flat, rose-colored spots. Internal bleeding and death can occur but are rare.

MORPHOLOGY OF SALMONELLA TYPHI (S. TYPHI)

Shape – *Salmonella typhi* is a rod shape (bacillus) bacterium.

Size – The size of *Salmonella typhi* is about 1–3 µm × 0.5–0.6 µm (micrometer).

Arrangement Of Cells – *Salmonella typhi* is arranged singly or in pairs.

Motility – *Salmonella typhi* is a motile bacterium.

Flagella – *Salmonella typhi* is a flagellated bacterium with peritrichous flagella arrangement.

Spores – The *Salmonella typhi* is a non–sporing bacterium.

Capsule – *S. typhi* is a non–capsulated bacterium.

Gram Staining Reaction – *Salmonella typhi* is a Gram -ve (Negative) bacterium.

CULTURE REQUIREMENTS OF SALMONELLA TYPHI (S. TYPHI)

⇒ ***Special requirements*** – *Salmonella typhi or S. typhi* have no complex nutritional requirements and readily grow in an ordinary media like Nutrient Agar medium (NAM). Commonly the NAM & MacConkey Agar medium is used for the cultivation of *Salmonella typhi* in Laboratory. For the isolation from feces, selenite F broth & XLD medium are commonly used.

⇒ ***Optimum temperature*** –The optimum temperature for the cultivation of *Salmonella typhi* in the laboratory is 37°C.

⇒ ***Optimum pH*** – *S. typhi* can survive at 4.1– 9.0 pH but the maximum growth observed around 6.5-7.5 i.e. slightly acidic to slightly alkaline pH. Also, the pH requirements vary as per the strain of *Salmonella typhi.*

⇒ ***Oxygen requirements*** – *Salmonella typhi (S. typhi*) is an ***aerobic*** bacterium i.e. grow best in the presence of oxygen and it is also a ***Facultative anaerobic*** organism i.e. can grow in the low oxygen environment.

⇒ There are various culture media used for the cultivation of *Salmonella typhi (S. typhi)* in the laboratory and most commonly the MacConkey Agar medium, XLD medium, and Selenite F broth medium is used which may vary as per the ***SOPs*** of the laboratory, the other media are as follows –

- Columbia Horse Blood Agar medium.
- Sheep Blood Agar medium.
- Eosin Methylene blue Agar (EMB) medium
- Deoxycholate Citrate Agar (DCA) medium (***Selective medium for Salmonella & Shigella***).
- Salmonella – Shigella Agar medium (***Selective medium for Salmonella & Shigella***).
- Wilson & Blair bismuth sulfite medium (***Selective medium for Salmonella***).
- Xylose Lysine Deoxycholate (XLD) medium (***Selective medium for Salmonella & Shigella***).
- Tetrathionate broth (***Selective medium***).
- Selenite F Broth (***Selective medium for Salmonella & Shigella***).
- The Liquid medium (Trypticase Soy Broth, Nutrient broth etc.)

WHAT CAUSES TYPHOID FEVER?

- The bacterium *Salmonella typhi* (*S. typhi*) causes typhoid fever. The bacteria spreads through contaminated food, drink, or water. People infected with *Salmonella typhi* carry the bacteria in their intestinal tract and blood.
- *Salmonella typhi* is shed (discarded from the body) in feces (stool). You may get typhoid fever if you ingest food or beverages prepared by someone who is shedding the bacteria and who does not wash their hands properly. In less developed countries, sewage containing *Salmonella typhi* may contaminate local water supplies.
- In some cases, people who have previously had typhoid fever still carry *Salmonella typhi* bacteria. These people are carriers of the disease. They may spread the infection even when they have no symptoms (the famous case of "Typhoid Mary" in the U.S.).

WHAT ARE THE SYMPTOMS OF TYPHOID FEVER?

In early stages of the disease, symptoms include: abdominal pain, fever, and a general feeling of being unwell. These initial symptoms are similar to other illnesses.

As typhoid fever gets worse, symptoms often include:

- High fever of up to 104 degrees Fahrenheit
- Headaches
- Abdominal pain, constipation then perhaps diarrhea later
- Small, red spots on your abdomen or chest (rose-colored spots)
- Loss of appetite and weakness

Other symptoms of typhoid fever include:

- Body aches
- Bloody stools
- Chills
- Severe fatigue
- Difficulty paying attention
- Agitation, confusion

HOW IS TYPHOID FEVER TREATED?

- Antibiotics are used to treat typhoid fever. These medications kill the bacteria that cause the infection. Several different types of antibiotics are used to treat typhoid fever. In many cases, typhoid fever is treated with ampicillin, chloramphenicol, or cotrimoxazole (Bactrim®). However, doctors also use fluoroquinolones (including Cipro® and Levaquin®), cephalosporins (including Cefepime®), and azithromycin.
- Your doctor will choose based on the most up-to-date recommendations. Antibiotics are widely available in the United States and in most other countries in the world. Do not attempt to self-treat with leftover antibiotics.
- Some people need supportive therapies, such as fluid or electrolyte replacement, depending on the severity of the infection.

WHAT COMPLICATIONS ARE ASSOCIATED WITH TYPHOID FEVER?

People who do not receive treatment for typhoid fever may have symptoms of the disease for months. In those cases, complications, like kidney failure or intestinal hemorrhage (severe bleeding), are possible. In severe cases, typhoid fever is fatal if left untreated. They may also become carriers and spread the illness to others.

BACTERIA

The Gram-negative bacterium that causes typhoid fever is *Salmonella enterica* subsp. enterica serovar Typhi. Based on MLST subtyping scheme, the two main sequence types of the *S.* Typhi are ST1 and ST2, which are currently widespread globally.The global phylogeographical analysis showed dominance of a haplotype 58 (H58) which probably originated in India during the late 1980s and is now spreading through the world carrying multidrug resistance.A more

detailed genotyping scheme was reported in 2016 and is now being used widely. This scheme re-classified the nomenclature of H58 to genotype 4.3.1.

CHOLERA

INTRODUCTION

Cholera is a rapidly dehydrating diarrheal disease caused by a toxin-producing bacteria, *Vibrio cholerae.*

MICROBIOLOGY

- **Etiologic agent** — *V. cholerae* is a distinctive, comma-shaped gram-negative rod. Organisms are highly motile and possess a single polar flagellum. *V. cholerae* is salt-tolerant, requiring NaCl for growth (halophilic) and exists naturally in aquatic environments.
- While in aquatic environments, *V. cholerae* may enter a viable but non-culturable form However, *V. cholerae* is readily grown from clinical specimens, including stool and rectal swabs, and can be identified in microbiology laboratories using selective media and biochemical tests. (See "Cholera: Clinical features, diagnosis, treatment, and prevention", section on 'Diagnosis'.)
- Only cholera toxin-producing (toxigenic) strains of *V. cholerae* are associated with cholera. While some environmental *V. cholerae* are toxigenic and capable of causing cholera, most environmental *V. cholerae* isolates are not toxigenic. Toxigenic strains harbor a filamentous bacteriophage (CTXΦ) which encodes cholera toxin

- The cause of cholera is infection by the *V. cholera* bacteria. These bacteria were discovered in 1883.
- The German bacteriologist, Robert Koch (1843-1910), studied the disease during an epidemic in Egypt. He found a bacterium in the intestines of those who had died of cholera but could neither isolate the organism nor infect animals with it.
- Later that year, Koch went to India, where he succeeded in isolating the bacteria. He discovered that they thrived in damp, dirty linen and moist earth, and in the stools of patients with the disease.

- *V. cholera* bacteria live in shallow, salty water on microscopic crustaceans. They can also existas colonies of biofilms that coat the surface of the water, plants, stones, shells, and similar items, and they can live among the eggs of midges, which serve as a reservoir for cholera bacteria.

- Toxic strains of cholera bacteria produce a poison that triggers violent diarrhea in humans.

COMPLICATIONS

Cholera can quickly become fatal. In the most severe cases, the rapid loss of large amounts of fluids and electrolytes can lead to death within hours. In less extreme situations, people who don't receive treatment can die of dehydration and shock hours to days after cholera symptoms first appear.

Although shock and severe dehydration are the worst complications of cholera, other problems can occur, such as:

- **Low blood sugar (hypoglycemia).** Dangerously low levels of blood sugar (glucose) — the body's main energy source — can occur when people become too ill to eat. Children are at greatest risk of this complication, which can cause seizures, unconsciousness and even death.
- **Low potassium levels.** People with cholera lose large quantities of minerals, including potassium, in their stools. Very low potassium levels interfere with heart and nerve function and are life-threatening.
- **Kidney failure.** When the kidneys lose their filtering ability, excess amounts of fluids, some electrolytes and wastes build up in the body — a potentially life-threatening condition. In people with cholera, kidney failure often accompanies shock.

PREVENTION

Cholera is rare in the United States with the few cases related to travel outside the U.S. or to contaminated and improperly cooked seafood from the Gulf Coast waters.

If you're traveling to areas known to have cholera, your risk of contracting the disease is extremely low if you follow these precautions:

- **Wash your hands with soap and water frequently,** especially after using the toilet and before handling food. Rub soapy, wet hands together for at least 15 seconds before rinsing. If soap and water aren't available, use an alcohol-based hand sanitizer.
- **Drink only safe water,** including bottled water or water you've boiled or disinfected yourself. Use bottled water even to brush your teeth.

  Hot beverages are generally safe, as are canned or bottled drinks, but wipe the outside before you open them. Don't add ice to your drinks unless you made it yourself using safe water.

- **Eat food that's completely cooked and hot** and avoid street vendor food, if possible. If you do buy a meal from a street vendor, make sure it's cooked in your presence and served hot.
- **Avoid sushi,** as well as raw or improperly cooked fish and seafood of any kind.
- **Stick to fruits and vegetables that you can peel yourself,** such as bananas, oranges and avocados. Stay away from salads and fruits that can't be peeled, such as grapes and berries.

When the bacteria enter areas where humans live, they can quickly cause severe epidemics. Weather changes, population loss, and improved sanitation can all end an outbreak.

CAUSES

- Cholera bacteria enter the body through the mouth, often in food or water that has been contaminated with human waste, due to poor sanitation and hygiene.

- They can also enter by eating seafood that is raw or not completely cooked, in particular shellfish native to estuary environments, such as oysters or crabs.

- Poorly cleaned vegetables irrigated by contaminated water sources are another common source of infection.

- In situations where sanitation is severely challenged, such as in refugee camps or communities with highly limited water resources, a single affected victim can contaminate all the water for an entire population.

Cholera vaccine

For adults traveling from the United States to areas affected by cholera, a vaccine called Vaxchora is available in the United States. It is a liquid dose taken by mouth at least 10 days before travel.

A few other countries offer oral vaccines as well. Contact your doctor or local office of public health for more information about these vaccines. Even with the vaccine, it's important to take the above precautions to prevent cholera.

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MALARIA

Malaria is a serious tropical disease spread by mosquitoes. If it isn't diagnosed and treated promptly, it can be fatal.

A single mosquito bite is all it takes for someone to become infected.

SYMPTOMS OF MALARIA

It's important to be aware of the symptoms of malaria if you're travelling to areas where there's a high risk of the disease. Symptoms include:

- a high temperature of 38C or above
- feeling hot and shivery
- headaches
- vomiting
- muscle pains
- diarrhoea

WHAT CAUSES MALARIA?

Malaria is caused by a type of parasite known as Plasmodium. There are many different types of Plasmodia parasites, but only 5 cause malaria in people.
The Plasmodium parasite is mainly spread by female Anopheles mosquitoes, which mainly bite at dusk and at night. When an infected mosquito bites a person, it passes the parasites into the bloodstream.
Malaria can also be spread through blood transfusions and the sharing of needles, but this is very rare.
Preventing malaria
Many cases of malaria can be avoided. An easy way to remember is the ABCD approach to prevention:

- **Awareness of risk** – find out whether you're at risk of getting malaria before travelling.
- **Bite prevention** – avoid mosquito bites by using insect repellent, covering your arms and legs, and using an insecticide-treated mosquito net.
- **Check whether you need to take malaria prevention tablets** – if you do, make sure you take the right antimalarial tablets at the right dose, and finish the course
- **Diagnosis** – seek immediate medical advice if you develop malaria symptoms, as long as up to a year after you return from travelling.

COMPLICATIONS OF MALARIA

Malaria is a serious illness that can get worse very quickly. It can be fatal if not treated promptly.

It can also cause serious complications, including:

- **severe anaemia** – where red blood cells are unable to carry enough oxygen around the body, leading to drowsiness and weakness
- **cerebral malaria** – in rare cases, the small blood vessels leading to the brain can become blocked, causing seizures, brain damage and coma

TYPES OF ANTIMALARIAL MEDICATION

The main types of antimalarials used to prevent malaria are described below.

Atovaquone plus proguanil

- **Dosage** – the adult dose is 1 adult-strength tablet a day. Child dosage is also once a day, but the amount depends on the child's weight. It should be started 1 or 2 days before your trip and taken every day you're in a risk area, and for 7 days after you return.
- **Recommendations** – a lack of clear evidence means this antimalarial shouldn't be taken by pregnant or breastfeeding women. It's also not recommended for people with severe kidney problems.
- **Possible side effects** – stomach upset, headaches, skin rash and mouth ulcers.
- **Other factors** – it can be more expensive than other antimalarials, so may be more suitable for short trips.

Doxycycline (also known as Vibramycin-D)

- **Dosage** – the dose is 100mg daily as a tablet or capsule. You should start the tablets 2 days before you travel and take them each day you're in a risk area, and for 4 weeks after you return.
- **Recommendations** – not normally recommended for pregnant or breastfeeding women, but your GP will advise. Not recommended for children under the age of 12 (because of the risk of permanent tooth discolouration), people who are sensitive to tetracycline antibiotics, or people with liver problems.
- **Possible side effects** – stomach upset, heartburn, thrush, and sunburn as a result of light sensitivity. It should always be taken with food, preferably when standing or sitting.
- **Other factors** – it is relatively cheap. If you take doxycycline for acne, it will also provide protection against malaria as long as you're taking an adequate dose. Ask your GP.

Mefloquine (also known as Lariam)

- **Dosage** – the adult dose is 1 tablet weekly. Child dosage is also once a week, but the amount will depend on their weight. It should be started 3 weeks before you travel and taken all the time you're in a risk area, and for 4 weeks after you get back.
- **Recommendations** – it's not recommended if you have epilepsy, seizures, depression or other mental health problems, or if a close relative has any of these conditions. It's not usually recommended for people with severe heart or liver problems.
- **Possible side effects** – dizziness, headache, sleep disturbances (insomnia and vivid dreams) and psychiatric reactions (anxiety, depression, panic attacks and hallucinations). It's very important to tell your doctor about any previous mental health problems, including mild depression. Don't take this medication if you have a seizure disorder.
- **Other factors** – if you haven't taken mefloquine before, it's recommended you do a 3-week trial before you travel to see whether you develop any side effects.

Chloroquine and proguanil

A combination of antimalarial medications called chloroquine and proguanil is also available, although these are rarely recommended nowadays because they're largely ineffective against the most common and dangerous type of malaria parasite, Plasmodium falciparum.

However, chloroquine and proguanil may occasionally be recommended for certain destinations where the Plasmodium falciparum parasite is less common than other types, such as India and Sri Lanka.

FUNGAL SKIN DISEASES

WHAT IS A SKIN INFECTION?

Your skin is the largest organ of your body. Its function is to protect your body from infection. Sometimes the skin itself becomes infected. Skin infections are caused by a wide variety of germs, and symptoms can vary from mild to serious. Mild infections may be treatable with over-the-counter medications and home remedies, whereas other infections may require medical attention. Read on to learn more about skin infections and what to do if you have one.

WHAT ARE THE TYPES OF SKIN INFECTIONS?

The following are four different types of skin infections:

1. Bacterial skin infections

Bacterial skin infections often begin as small, red bumps that slowly increase in size. Some bacterial infections are mild and easily treated with topical antibiotics, but other infections require an oral antibiotic. Different types of bacterial skin infections include:

- cellulitis
- impetigo
- boils
- leprosy

2. Viral skin infections

Viral skin infections are caused by a virus. These infections range from mild to severe. Different types of viral infections include:

- shingles (herpes zoster)
- chickenpox
- Molluscum contagiosum
- warts
- measles
- hand, foot, and mouth disease

3. Fungal skin infections

These types of skin infections are caused by a fungus and are most likely to develop in damp areas of the body, such as the feet or armpit. Some fungal infections aren't contagious, and these infections are typically non-life-threatening.

Different types of fungal infections:

- athlete's foot
- yeast infection
- ringworm
- nail fungus
- oral thrush
- diaper rash

4. Parasitic skin infection

These types of skin infections are caused by a parasite. These infections can spread beyond the skin to the bloodstream and organs. A parasitic infection isn't life-threatening but can be uncomfortable.

Different types of parasitic skin infections include:

- lice
- bedbugs
- scabies
- cutaneous larva migrans

WHAT IS A FUNGAL SKIN INFECTION?

- ❖ Fungi live everywhere. They can be found in plants, soil, and even on your skin. These microscopic organisms on your skin typically don't cause any problem, unless they multiply faster than normal or penetrate your skin through a cut or lesion.
- ❖ Since fungi thrive in warm, moist environments, fungal skin infections can often develop in sweaty or damp areas that don't get much airflow. Some examples include the feet, groin, and folds of skin.
- ❖ Often, these infections appear as a scaly rash or discoloration of the skin that is often itchy.
- ❖ Some fungal skin infections are very common. Although the infection can be annoying and uncomfortable, it's typically not serious.
- ❖ Fungal skin infections are often spread through direct contact. This can include coming into contact with fungi on clothing or other items, or on a person or animal.

WHAT ARE THE MOST COMMON FUNGAL SKIN INFECTIONS?

- ❖ Many common fungal infections can affect the skin. In addition to the skin, another common area for fungal infections is mucous membranes. Some examples of these are vaginal yeast infections and oral thrush.
- ❖ Below, we'll explore some of the most common types of fungal infections that can impact the skin.
- ❖ Ringworm of the body (tinea corporis)
- ❖ Contrary to its name, ringworm is caused by a fungus and not a worm. It typically occurs on the torso and limbs. Ringworm on other areas of the body can have different names, such as athlete's foot and jock itch.
- ❖ The main symptom of ringworm is a ring-shaped rash with slightly raised edges. The skin inside these circular rashes usually looks healthy. The rash can spread and is often itchy.
- ❖ Ringworm is a common fungal skin infection and is highly contagious. It's not serious, though, and can usually be treated with an antifungal cream.

ATHLETE'S FOOT (TINEA PEDIS)

Athlete's foot is a fungal infection that affects the skin on your feet, often between your toes. Typical symptoms of athlete's foot include:

- itching, or a burning, stinging sensation between your toes or on the soles of your feet
- skin that appears red, scaly, dry, or flaky
- cracked or blistered skin

In some cases, the infection can also spread to other areas of your body. Examples include your nails, groin, or hands (tinea manuum).

JOCK ITCH (TINEA CRURIS)

Jock itch is a fungal skin infection that happens in the area of your groin and thighs. It's most common in men and adolescent boys.

The main symptom is an itchy red rash that typically starts in the groin area or around the upper inner thighs. The rash may get worse after exercise or other physical activity and can spread to the buttocks and abdomen.

The affected skin may also appear scaly, flaky, or cracked. The outer border of the rash can be slightly raised and darker.

RINGWORM OF THE SCALP (TINEA CAPITIS)

This fungal infection affects the skin of the scalp and the associated hair shafts. It's most common in young children and needs to be treated with prescription oral medication as well as antifungal shampoo. The symptoms can include:

- localized bald patches that may appear scaly or red
- associated scaling and itching
- associated tenderness or pain in the patches

TINEA VERSICOLOR

- ❖ Tinea versicolor, sometimes called pityriasis versicolor, is a fungal/yeast skin infection that causes small oval discolored patches to develop on the skin. It's caused by an overgrowth of a specific type of fungus called *Malassezia*, which is naturally present on the skin of about 90 percent of adults.
- ❖ These discolored skin patches most often occur on the back, chest, and upper arms. They may look lighter or darker than the rest of your skin, and can be red, pink, tan, or brown. These patches can be itchy, flaky, or scaly.
- ❖ Tinea versicolor is more likely during the summer or in areas with a warm, wet climate. The condition can sometimes return following treatment.

CUTANEOUS CANDIDIASIS

This is a skin infection that's caused by *Candida* fungi. This type of fungi is naturally present on and inside our bodies. When it overgrows, an infection can happen.

Candida skin infections occur in areas that are warm, moist, and poorly ventilated. Some examples of typical areas that can be affected include under the breasts and in the folds of the buttocks, such as in diaper rash.

The symptoms of a *Candida* infection of the skin can include:

- a red rash
- itching
- small red pustules

ONYCHOMYCOSIS (TINEA UNGUIUM)

Onychomycosis is a fungal infection of your nails. It can affect the fingernails or the toenails, although infections of the toenails are more common.

You may have onychomycosis if you have nails that are:

- discolored, typically yellow, brown, or white
- brittle or break easily
- thickened

Prescription medications are often required to treat this type of infection. In severe cases, your doctor may remove some or all of an affected nail.

SKIN FUNGUS TREATMENT

Antifungal medications work to treat fungal infections. They can either kill fungi directly or prevent them from growing and thriving. Antifungal drugs are available as OTC treatments or prescription medications, and come in a variety of forms, including:

- creams or ointments
- pills
- powders
- sprays
- shampoos

PREVENTION

Try to keep the following tips in mind to help prevent a fungal skin infection from developing:

- Be sure to practice good hygiene.
- Don't share clothing, towels, or other personal items.
- Wear clean clothes every day, particularly socks and underwear.
- Choose clothing and shoes that breathe well. Avoid clothing or shoes that are too tight or have a restrictive fit.
- Make sure to dry off properly with a clean, dry, towel after showering, bathing, or swimming.
- Wear sandals or flip-flops in locker rooms instead of walking with bare feet.
- Wipe down shared surfaces, such as gym equipment or mats.
- Stay away from animals that have signs of a fungal infection, such as missing fur or frequent scratching.