STUDY MATERIAL
II SEMESTER
COURSE 3: - INTRODUCTION TO MICROBIOLOGY

Unit - 1: History of Microbiology

Q. Charaka Samhita (4 Marks)

Aseptic techniques with reference to Charaka Samhita

Charaka Samhita is one of the most ancient texts in the field of Ayurveda. It emphasized the following points-

1. **Personal hygiene** - it emphasizes the importance of personal cleanliness for both the physician and the patient. It includes regular bathing, washing hands thoroughly and wearing clean clothes to prevent the transmission of diseases.
2. **Environmental cleanliness** - its stresses the importance of keeping the treatment area and the surrounding clean and free from contamination, maintaining proper sanitation and hygienic environment for successful medical practices
3. **Purity of medicines and ingredients**- it highlights importance of using pure and contaminated medicines and ingredients in treatments to ensure the effectiveness and safety
4. **Sterilization of instruments** – it includes techniques for disinfecting instrument to eliminate harmful microorganisms. it includes burning, heating or using certain medical preparation to achieve the desire effect .
5. **Preventing cross contamination** during treatment - it this includes using separate containers for different patients and proper cleaning instrument between uses
6. **Wound care & Wound Management** – it includes surgical procedures and proper wound care like cleaning and dressing wound.
7. **Quarantine & isolating** – it includes isolation of patience with infectious diseases to prevent spread of infection to others.

Q. EDWARD JENNER (4 Marks)

Edward Jenner was an English country doctor who introduced the vaccine for *smallpox*. Jenner is often called "the father of immunology"
Smallpox is a deadly disease caused by the **variola virus**. It causes painful lesions that leave disfiguring scars on the skin of people who survive and can also cause blindness. It caused horrible facial disfigurement

It was also common knowledge that dairymaids were often immune to smallpox.

This was because they were exposed to **cowpox**, a similar virus that affects cows.

In humans, it causes a much milder version of smallpox. If the dairymaids got cowpox, they would be immune to smallpox afterwards, because the antibodies their bodies had made against the cowpox virus would also work against the smallpox virus.

The story involves a dairymaid called Sarah Nelmes, an eight-year-old boy called James Phipps and a cow known as Blossom.

Sarah came to Jenner in **1796** with a rash on her right hand and he diagnosed .

Sarah told Jenner that one of cow called Blossom had recently been infected with cowpox.

Sarah's pustules were on the part of the right hand that handled the animal's teats.

Jenner took some pus from Sarah's hand and scratched it into the arm of young *James Phipps*s, the son of his gardener.

The lad became mildly ill but recovered, confirming that cowpox can be transmitted from person to person.

Several days later, Jenner exposed the boy to smallpox. Much to Jenner's relief, the boy survived and . He was found to be immune.
this known as vaccination – a name taken from the Latin word vacca, meaning a cow.

Jenner called his new method '*vaccination*' after the Latin word for cow
In 1853, 30 years after Jenner's death, smallpox vaccination was made compulsory in England and Wales.

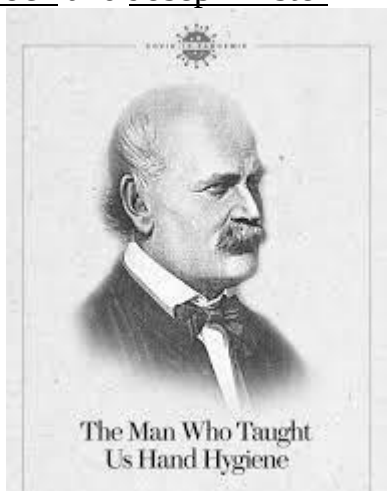
Q. Ignaz Semmelweis (4 Marks)

Ignaz Semmelweis was the first doctor to discover the importance of **HAND WASHING** for medical professionals.
In the 19th century, it was common for women to die from an illness contracted during or after childbirth, known as childbed fever.
Semmelweis worked at an obstetric department in Vienna General hospital, Austria. He noticed that women delivered by physicians and medical students in ward one had a much higher death rate than women delivered by midwives in ward 2 of the hospital.
He observed medical students performed autopsies on dead bodies and then directly carried out vaginal examinations on living patients without hand washing. He suspected that the students carried disease from the dead bodies to the patients they examined. Although Semmelweis did not know the true cause of puerperal fever, he proposed that physicians were somehow transferring the causative agent to their patients.

He suggested that the number of puerperal fever cases could be reduced if physicians and medical students simply washed their hands with chlorinated lime water before and after examining every patient.

After Semmelweis started compulsory hand-washing, the death rate for women in ward 1 reduced. When this practice was implemented, the maternal mortality rate in ward 1 decreased and became same as ward 2.

This demonstrated that handwashing was a very effective method for preventing disease transmission.
The younger medical men in Vienna recognized the significance of Semmelweis's discovery and gave him all possible assistance. His superior, on the other hand, was critical because he failed to understand him.
His work was only widely appreciated two decades later, after research by Louis Pasteur, Robert Koch and Joseph Lister



Q. LOUIS PASTEUR (4 Marks)

Louis Pasteur was a French microbiologist and chemist . He is known for his discoveries of rabies ,anthrax ,chicken cholera vaccines , microbial fermentation and pasteurization. Louis Pasteur is regarded as the father of Modern Microbiology.

GERM THEORY OF FERMENTATION
Pasteur proved that fermentation is caused by a microorganism. Using this knowledge he saved French wine industry.

GERM THEORY OF DISEASE
Germ theory states that many diseases were caused by the presence of microorganisms .He discovered various infectious diseases such as staphylococcus, streptococcus and pneumococcus

BIOGENESIS
Disapproved Spontaneous generation theory and gave law of biogenesis. He rejected the theory of spontaneous generation by his swan neck experiment. He proposed that life existed from pre-existing life

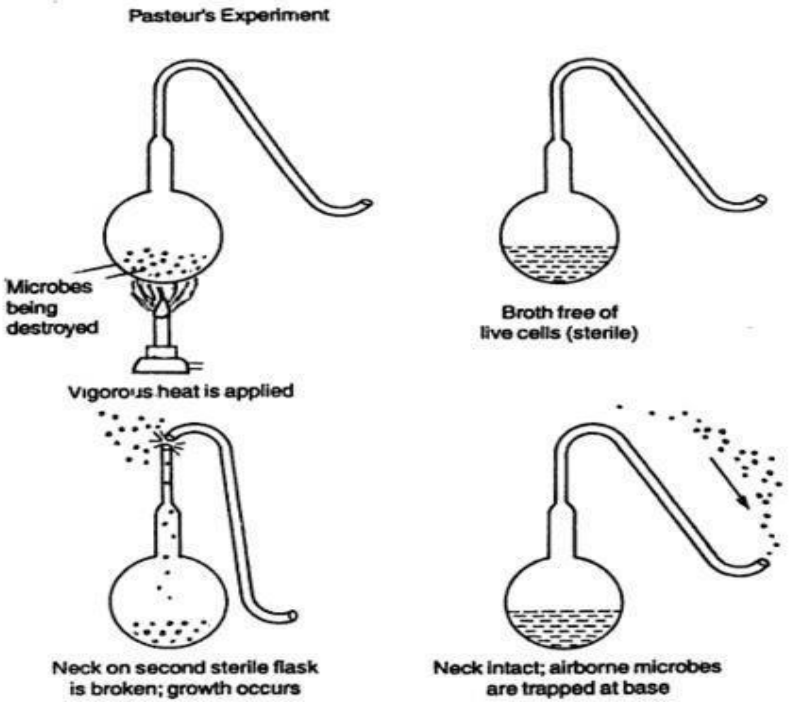
PASTEURISATION
He gave the pasteurization technique of food preservation. First it was for wine and beer later it was applied for milk preservation . It involved heating at 55 degree Celsius. This process was named as pasteurisation.

RABIES VACCINE
He discovered anti rabies vaccine. It was first used for Joseph Meister, a boy bitten by a rabid animal. The boy survived . the rabies vaccine became a success.

PEBRINE SILKWORM DISEASE
He worked on protecting the Silk Industry. He studied that infection was transmitted by parasites and showed how infected worms should be isolated and destroyed.

CHICKEN CHOLERA VACCINE
He discovered Chicken Cholera Vaccine.

ANTHRAX VACCINE
Anthrax is a disease caused by *Bacillus anthracis*. This bacteria was discovered by Robert Koch. Louis made the vaccination for anthrax


Pasteur's Experiment
Microbes being destroyed
Vigorous heat is applied
Broth free of live cells (sterile)
Neck on second sterile flask is broken; growth occurs
Neck intact; airborne microbes are trapped at base

Q. ROBERT KOCH **(4 Marks)**

Robert Koch was a German doctor. He is known as the founder of bacteriology and microbiology. He investigated the anthrax disease cycle, and studied the bacteria that cause tuberculosis, and cholera. He also formulated Koch's postulates. Koch won the 1905 Nobel Prize in Medicine.

- Anthrax: - he discovered the causative agent of anthrax disease Bacillus anthracis. It killed many cattles.
- Cholera bacteria: - he discovered the causative agent of Cholera disease as Vibrio cholerae .
- Tuberculosis :-Koch showed that tuberculosis was caused by Mycobacterium tuberculosis.
- Pure culture technique:-he developed methods of isolating bacteria in pure culture by using solid nutrition medium.
- Staining :- Koch and his team also developed ways of *staining* bacteria to improve the bacteria's visibility under the *microscope*,
- Nutrient broth and nutrient agar:-He used gelatin a solidifying agent in his many experiments . Dr.Walter Hesse and his wife Fanny used agar insted of gelatine which is used still today in microbiology lab.
- Petri dishes :- And his assistant Petri introduced Petri dishes in 1887 which are still used in microbiology labs all over the world.

His research led to the creation of Koch's postulates .These are as follows:-

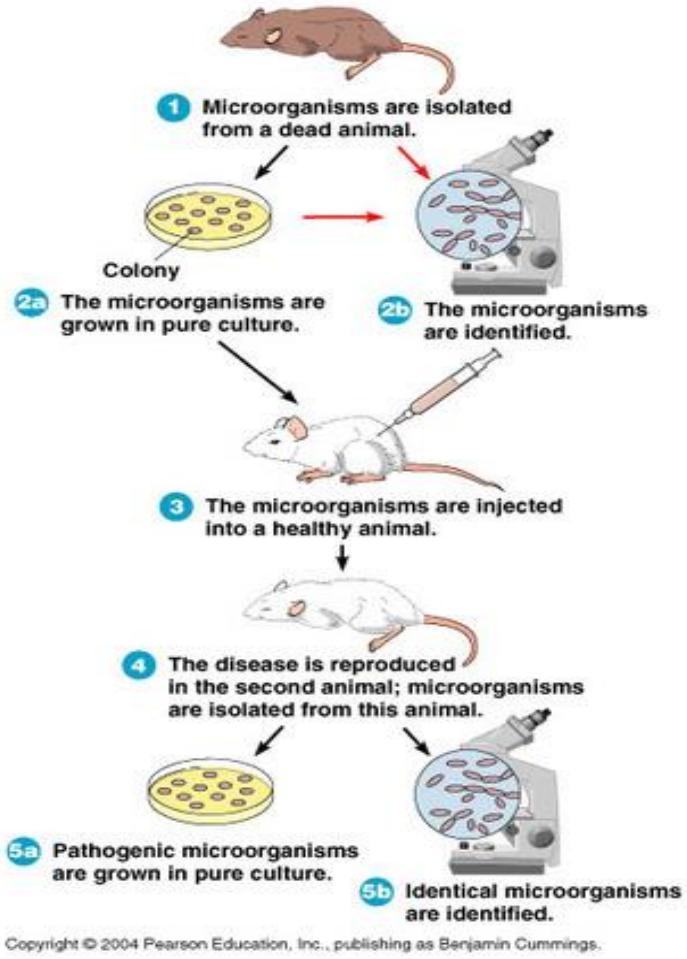
Koch's Postulates

1. Microorganisms are isolated from dead animals
2. Microorganisms are grown in pure culture

2b. Microorganisms are identified

3. Microorganisms are injected into healthy animals
4. Disease is reproduced in second animal
5. Microorganisms are grown in pure culture

5b. Identification of identical microorganism.



Koch postulates

Q. JOSEPH LISTER "Father of Antiseptic Surgery" (4 Marks)

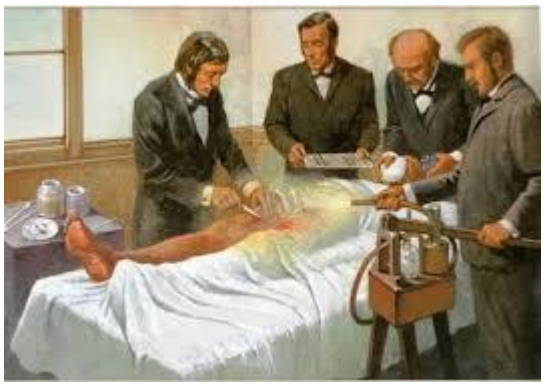
- Joseph Lister was **born** in England on 5 April **1827**.
- His father taught him how to use a microscope
- By the age of **16** he wanted to be a surgeon.
- Lister was shocked that **half of patients died** after surgery.
- He learned about **invisible germs** from Louis Pasteur's work
- Lister began experimenting with chemicals to clean patients' wounds.
- Cleaning wounds and surgical instruments with **antiseptic** made the **survival rate higher**.
- Lister began persuading others to use the same methods.
- Joseph Lister **died** in England on 10 February **1912** aged **87**.

Surgery before Lister

- Surgery was very dangerous and the **high death rate** made many people suggest it should be stopped.
- Patients often died from infections after surgery.
- Surgeons wore their **bloody** and **unwashed medical clothes** during surgery.
- Medical instruments were **rarely cleaned** and staff **never washed their hands**.

Lister's experiments

- Lister thought **germs caused infection**
- He soaked bandages in **carbolic acid** to keep wounds clean. It was normally used for cleaning sewers!
- Patients began to heal properly with Lister's **new antiseptic treatment**
- Lister decided that **hands, clothes, surgical tools, and wounds** should also be washed with this chemical.
- This led the way for other types of surgery due to **less risk of infection**.
- Lister even invented a carbolic acid spray machine to clear **surgical theatres**.
- Lister's carbolic acid steam spray was used to kill germs



ANTISEPTIC SURGERY USINF CARBOLIC ACID SPRAY

Unit - 2: Place of Microorganisms in the living world

Q. Five Kingdom Classification System (8 Marks)

Robert Harding Whittaker was an American plant ecologist. The **five kingdom classification** was proposed by R.H. **Whittaker** in 1969. The **five kingdoms** were formed on the basis of characteristics such as

- cell structure
- mode of nutrition
- source of nutrition
- body organisation.

It includes **Kingdom** Monera,**Kingdom** Protista, **Kingdom** Fungi, **Kingdom** Plantae, and **Kingdom** Animalia.

Once upon a time, all living things were classified into two kingdoms, namely plants and animals .

Animals included every living thing that moved, ate, and grew to a certain size and stopped growing.

Plants included every living thing that did not move or eat and that continued to grow throughout life.

It became very difficult to group some living things into one or the other, so the two kingdoms were expanded into five kingdoms:

- Protista (the single-celled eukaryotes)
- Fungi (fungus and related organisms)
- Plantae (the plants)
- Animalia (the animals)
- Monera (the prokaryotes).

Taxonomic system is like this

- **Kingdom**
- **Phylum**
- **Class**
- **Order**
- **Family**
- **Genus**
- **Species**

Monera (includes Eubacteria and Archeobacteria)

- Individuals are single-celled, may or may not move, have a cell wall, have no chloroplasts or other organelles, and have no nucleus.
- Monera are usually very tiny, although one type, namely the blue-green bacteria, look like algae.
- They are filamentous and quite long, green, but have no visible structure inside the cells.
- No visible feeding mechanism.

- They absorb nutrients through the cell wall or produce their own by photosynthesis.

Protista

- Protists are single-celled and usually move by cilia, flagella, or by amoeboid mechanisms.
- There is no cell wall.
- They have organelles including a nucleus and may have chloroplasts.
- Nutrients are acquired by photosynthesis, ingestion of other organisms, or both.

Fungi

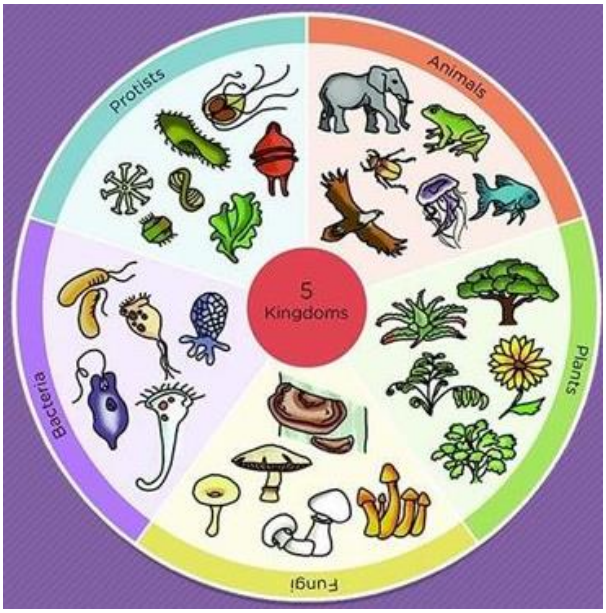
- Fungi are multicellular,with a cell wall, organelles including a nucleus, but no chloroplasts.
- They have no mechanisms for locomotion.
- Fungi range in size from microscopic to very large (such as mushrooms).
- Nutrients are acquired by absorption. Mostly fungi acquire nutrients from decaying material.

Plantae

- Plants are multicellular and most don't move
- Organelles including nucleus, chloroplasts are present, and cell walls are present.
- Nutrients are acquired by photosynthesis (they all require sunlight).

Animalia

- Animals are multicellular, and move with the aid of cilia, flagella, or muscular organs
- They have organelles including a nucleus, but no chloroplasts or cell walls.
- Animals acquire nutrients by ingestion.



Q. DIFFERENT BRANCHES OF MICROBIOLOGY (8 Marks)

Basic branches of microbiology

- **Bacteriology**: It is the study of bacteria.
- **Immunology**: It is the study of the immune system. It studies the relationships between pathogens such as bacteria and viruses and their hosts.
- **Mycology**: It is the study of fungi, such as yeasts and molds.
- **Nematology**: It is the study of nematodes (roundworms).
- **Phycology**: It is the study of algae.
- **Protozoology**: It is the study of protozoa, single-celled organisms likes amoebae.
- **Virology**: It is the study of viruses. Virologists study diseases and infections caused by viruses. This is a great scope in this area to study as the whole world experienced the pandemic COVID-19 in 2020.

Applied Branches of Microbiology - or - Applications of Microbiology

- **Agricultural microbiology**: it deals with the study of microorganisms that causes plant diseases such as TMV, Citrus Canker, Smut, Rust and methods of protection against such diseases. It also deals with microorganisms that improve soil fertility such as Rhizobium, Cyanobacteria. It also deals with the use of Bio Fertilizers and Bio Pesticides.
- **Food microbiology**: it deals with the study of Microorganisms used to make Bread, Alcohol, Beer, Yoghurt, Wine , Cheese, Curd, Kefir. It also deals with the study of Microorganisms that spoil food or cause food borne illnesses or food intoxication.
- **Medical microbiology**: it deals with the study of microorganisms responsible for human disease. Ex are AIDS from HIV, Typhoid, and Tuberculosis.
- **Microbial biotechnology**: it deals with the study of microorganisms used in genetic engineering for the production of insulin, growth hormones, bioactive molecules such as Streptokinase Cyclosporin A, statins
- **Pharmaceutical microbiology**: it deals with the study of microorganisms used in pharmaceutical products such as Vaccines Antibiotics, Interferons, Probiotics, Supplements, and Vitamins. Ex are Polio Vaccine, Penicillin, Streptomycin, and Vitamin B12 Etc.
- **Clean Energy**-Microorganisms are used to produce Bio ethanol and in Biogas. Bacteria are used to convert various forms of agricultural and urban waste into bio fuels such as Bio ethanol. Algae are used to make Biodiesel.

- **Industrial microbiology**-Microorganisms are used to produce many industrial chemicals and enzymes and other bioactive molecules such as Acetic Acid, Butyric Acid ,Lactic Acid , Citric Acid and enzymes like Amylases, Protease,Streptokinsae etc
- **Soil microbiology**-it deals with the study of Microbes that make nutrients and minerals in the soil available to plants, microbes that produce hormones that increase growth and reduce stress responses.
- **Aquatic microbiology**- it deals with the study of water purification, water borne diseases. It also deals with Water treatment. Microorganisms oxidise organic matter in waste water and clean the water. They are used in Biological Waste Water Treatment.

- **Geo microbiology**: - it deals with the microorganisms which are used in the production of Minerals and Metals from low grade ores. This is called as Bio Mining or Bioleaching.
- **Environmental microbiology: -** microorganisms are used to clean polluted water from industries and urban areas and also in Oil Biodegradation. Petroleum oil is toxic and it causes environmental pollution along coastal regions. Hydrocarbon-degrading activities of microbial communities help in cleaning and reduce pollution. Microbes also help in degrading many toxic chemicals.

Q. DIFFERENCE BETWEEN PROKARYOTIC & EUKARYOTIC CELLS (8 Marks)

Prokaryotic cells are cells without a nucleus. A prokaryote is a single-cell organism whose cell lacks a nucleus and other membrane-bound organelles. Prokaryotes are divided into two groups: the bacteria and the Achaea. Prokaryotes are small, single-celled and have a simple structure.

Eukaryotic cells are cells that contain a nucleus and other membrane-bound organelles like Mitochonria, Endoplasmic reticulum, Golgi complex, Lysosomes etc. Examples of eukaryotes include protozoa, fungi, plants and animals.

	Prokaryotes	Eukaryotes
Type of Cell	Always unicellular	Unicellular and multi-cellular
Cell size	Ranges in size from 0.2 μm – 2.0 μm in diameter	Size ranges from 10 μm – 100 μm in diameter
Cell wall	Mostly present; chemically complex in nature	When present, chemically simple in nature
Nucleus	In this cells true nucleus absent, instead nucleoid is present	True nucleus is present.
Ribosomes	Present. Smaller in size and spherical in shape	Present. Comparatively larger in size and linear in shape
DNA	Circular	Linear
Mitochondria	Absent	Present
Cytoplasm	Present, but cell organelles absent	Present, cell organelles present
Endoplasmic reticulum	Absent	Present
Plasmids	Present	Very rarely found in eukaryotes
Ribosome	Small ribosomes	Large ribosomes
Lysosome	Lysosomes and centrosomes are absent	Lysosomes and centrosomes are present
Cell division	Through binary fission	Through mitosis
Flagella	The flagella are smaller in size	The flagella are larger in size
Reproduction	Vegetative & Asexual	Both asexual and sexual
Example	Bacteria and Archaea	Plant and Animal cell

Unit - 3: Prokaryotic microorganisms and Viruses

Q. EXPLAIN GENERAL CHARACTERS OF BACTERIA (8 Marks)

Bacteria are single celled microbes. They have prokaryotic cell structure. They do not have nucleus and other cell organelles. The genetic information is contained in a single loop of DNA. Some bacteria have an extra circle of genetic material called a plasmid.

Habitat

Bacteria are found in everywhere on Earth: Soil, Rock, Oceans and even arctic snow. Some live on the skin of animals and humans. Most are found in the large intestine of animals.

Bacteria come in a wide variety of shapes. They are

- Coccus (circle or spherical)
- Bacillus (rod-like)
- Coccobacillus (between a sphere and a rod)
- Spiral (corkscrew-like)
- filamentous (elongated)

They can exist as single cells, in pairs, chains or clusters.

Bacteria Reproduction

Bacteria single-celled prokaryotic organisms. They mostly reproduce by binary fission.

Binary Fission

Bacteria reproduce by binary fission. In this process the bacterium, which is a single cell, divides into two identical daughter cells.

Some bacteria can acquire new DNA in three different ways:

- **Conjugation**
- **Transformation**
- **Transduction**.

Nutrition of bacteria:-

Photoautotrophs:

These bacteria capture the energy of sun light and transform it into the chemical energy. In this process CO_2 is reduced to carbohydrates e.g., Cyanobacteria.

Photoheterotrophs

These bacteria use light energy but cannot use carbon dioxide as their sole caron source instead they use organic compounds like carbohydrates, fatty acids, and alcohols . For e.g., Purple non-sulphur bacteria.

Chemoautotrophs:
They use inorganic energy sources such as hydrogen sulfide, elemental sulfur, ferrous iron, molecular hydrogen, and organic compounds.They can use carbon dioxide as their sole carbon source. Example- Sulphur oxidising bacteria, Nitorgen fixing bacteria.

Chemoheterotrophs:
These bacteria obtain both carbon and energy from organic compounds such as carbohydrates, lipids and proteins. Ex are E.coli, Bacillus.

Oxygen requirement of bacteria:-

- **Aerobic** bacteria require oxygen for growth.
- **Facultative anaerobes** grow in the presence or absence of oxygen.
- **Anaerobic bacteria** grow in the absence of oxygen.
- **Microaerophilic** bacteria grow best in the presence of low oxygen tension

Temperature requirement of Bacteria :

- Mesophiles: Mesophiles are the bacteria that survive at normal temperatures,e.g. *Staphylococcus aureus and E. coli.*
- Thermophiles: They can tolerate high range of temperatures,e.g. *Thermus aquaticus*.
- Psychrophiles: They are cold-loving microbes,e.g. *Arthrobacter* species.
- Hyperthermophiles: They can survive a range of temperatures of 80°-113°C,e.g. *Pyrolobus fumarii.*

pH requirement of bacteria:-

- **Neutrophiles –** these Bacteria grow best at neutral Ph close to 7.0
- **Acidophiles** grow best at a pH near 3.0.
- **Alkaliphiles** grow best at pH above 9

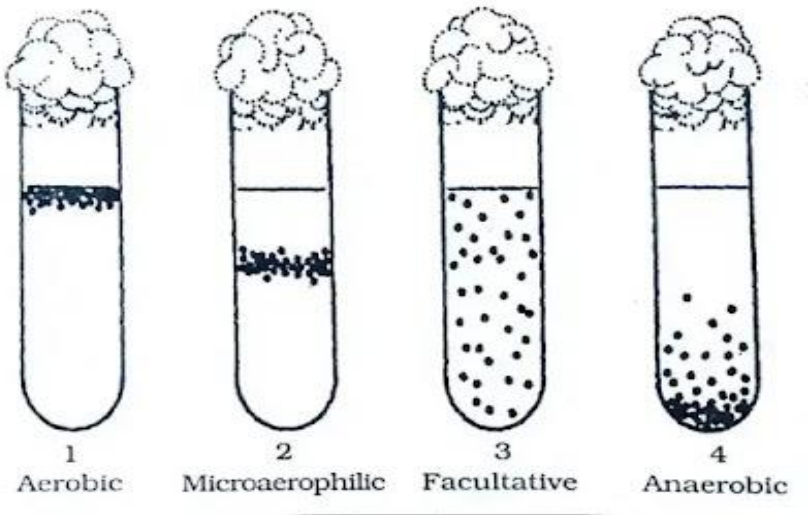
Depending on cell wall structure they are classified as Gram negative & Gram positive

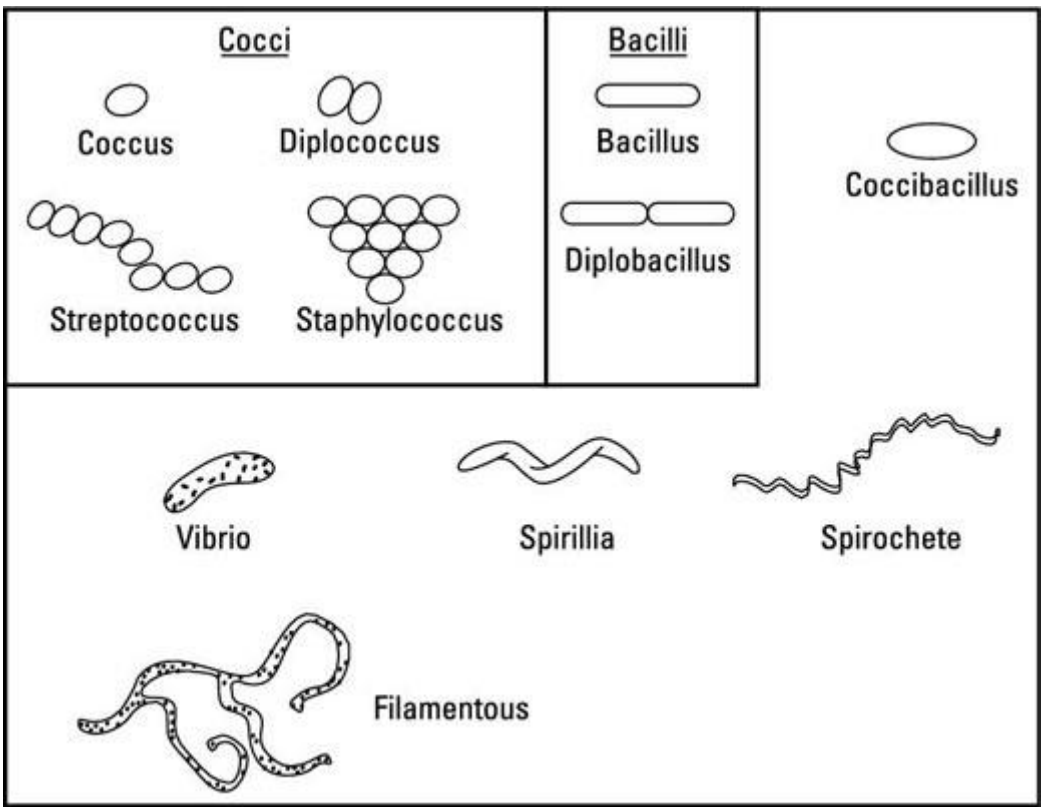
Example of Gram positive –

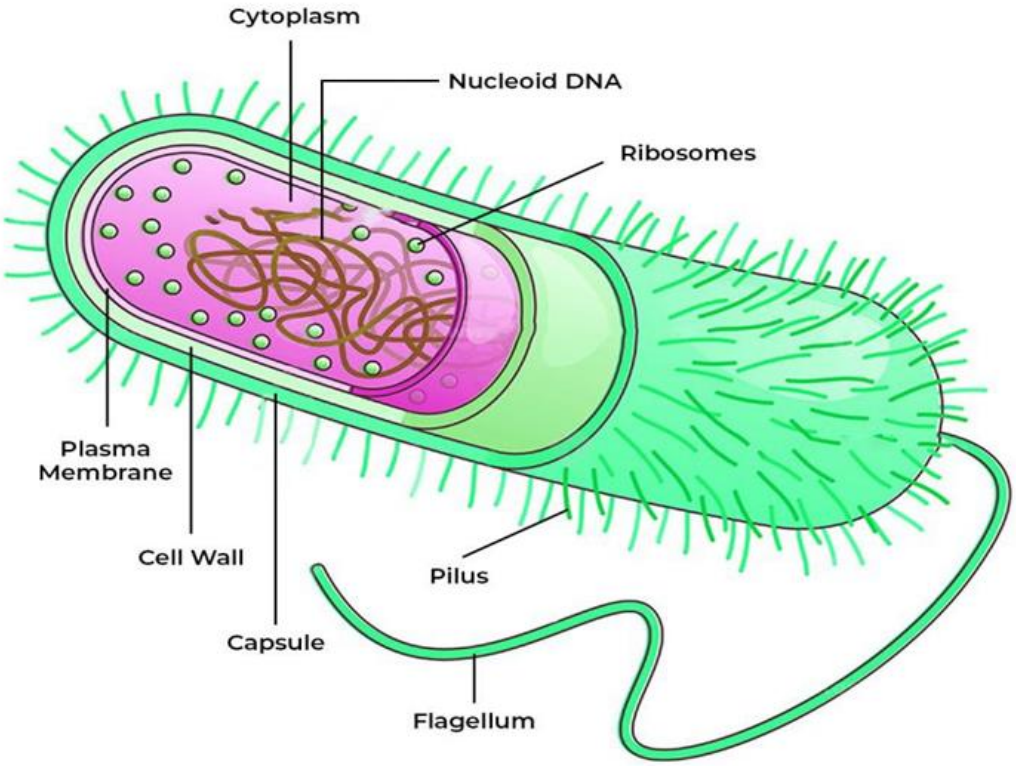
- *Bacillus anthracis*
- *Staphylococcus aureus*

Example of Gram negative –

- *E.Coli*
- *Salmonella typhi*






Cytoplasm
Nucleoid DNA
Ribosomes
Plasma Membrane
Cell Wall
Capsule
Pilus
Flagellum

Q.DISTINGUISHING CHARACTERS OF ARCHAEBACTERIA & EUBACTERIA (8 Marks)

Archaebacteria are one of the oldest living organisms on Earth. They are classified as bacteria because many of their features resemble the bacteria . They belong to the kingdom Archaea and hence are named Archaebacteria.

- Archaebacteria are ancient group of bacteria living in extreme environments.
- They are characterized by possessing cell walls without peptidoglycan.
- The lipids in their plasma membrane are branched differing from all other organisms.
- They are categorized into methanogens, halophiles and thermoacidophiles.

Archaebacteria	Eubacteria
Archaebacteria are primitive or ancient	Eubacteria are true bacteria
Archaebacteria have a variety of shapes and sizes, including spheres, rods, plates, spirals, flats, and squares.	Cocci, bacilli, vibrio, rods, filaments, and spirochetes are all examples of shapes of Eubacteria.
The organisation of Archaebacteria is simple.	Eubacteria includes more complicated organisms than archaebacteria.
Cell type is Prokaryotic	Cell type is Prokaryotic
Habitat is often extreme environments as well as normal. Example hot springs, hydrothermal vents, salt lakes, sewage digesters, and the rumen.	Habitat is often normal like soil, water air etc. and rarely in extreme environment
Unicellular & No membrane bound cell organelles	Unicellular &No membrane bound cell organelles
Cell wall are made up of Pseudo Peptidoglycans .	Cell wall are made up of peptidiglycan.
Cell membrane lipids are ether-linked, branching, aliphatic chains that include D-glycerol phosphate.	Cell membrane lipids are ester-linked, straight chains of fatty acids with L-glycerol phosphates.
Example are Methanogens, halophiles, and thermophiles	Examples of Eubacteria: gram - positive bacteria and gram- negative bacteria.
They reproduce asexually such as	They reproduce asexually through binary

through binary fission, budding, and fragmentation.	fission, budding, and fragmentation .
Examples of some genus of Archaebacteria are: *Halobacterium*, *Thermoplasma*.	Eubacteria includes bacteria such as *E.Coli*, *Bacillus*.

Q. GENERAL CHARACTERISTICS OF VIRUSES (8 Marks)

Viruses are the smallest of all the microbes. Viruses do not have cell structure. Viruses lack their own metabolism and grow only inside the host cell. So they are also called as **obligate intracellular parasites**. Viruses are found in almost everywhere on Earth. The study of viruses is known as **virology**. Viruses can infect animals and plants, microorganisms.

Dmitri Ivanovsky's from Russia and Martinus Beijerinckin from Holland together discovered the Tobacco Mosaic Virus.

STRUCTURE OF VIRUS

A virus is made up of either DNA or RNA. It is surrounded by a protective coat called a **Capsid** which is made up of protein. In some viruses the capsid is covered by an outer coat. It is called as **Envelope**.
The viral particles are also known as virions. It consists of the following:

- DNA or RNA. It carries genetic information
- A protein coat, called the capsid
- An envelope of lipids that surrounds the protein coat

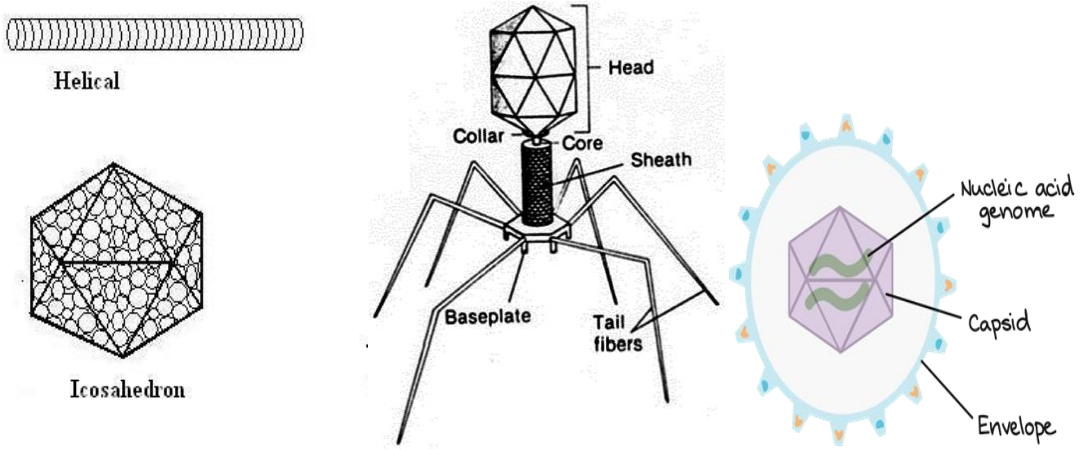
DNA viruses
DNA viruses use DNA as their genetic material. Examples of DNA viruses are Lambda virus, T2, T4, and Herpesvirus.

RNA viruses
The virus that possesses RNA as genetic material are called RNA viruses. Examples- Corona Virus, Polio Virus, Hepatitis C Virus, Rabies Virus, and HIV

SIZE
Viruses are much smaller than bacteria. They are sub microscopic. Virus are very small. Their size ranges from 20-300nm in diameter.

SHAPES OF VIRUSES
Viruses have four shapes
Helical - Helical viruses are long thread like in shape. They consist of nucleic acid. it is surrounded by a protein cylinder or capsid . Example TMV
PolyhedraL viruses consist of nucleic acid surrounded by a polyhedral (many-sided) shell or capsid. it is in the form of an icosahedron. eg. Adenovirus, Picornavirus, Papovavirus, herpes virus etc.
Spherical / Enveloped- Enveloped viruses are round in shape. They consist of nucleic acid surrounded aprotein coat. It is covered by envelope. The envelope is made up of Lipid. Herpes virus, Corona virus, HIV

Complex (Head & Tail) -they have complex structures with head and tail. For example a bacteriophage has polyhedral head with a helical tail. Ex- Pox virus, Bacteriophage, T-phage (T2,T4)



Based on the type of host, there are four different types of viruses:

Animal Viruses -These viruses infect the animal cells. Examples- Rabies Virus, Poliovirus, Herpes virus, HIV, Corona virus etc.

Plant Viruses -These viruses infect the cells of plants. Examples -Potato Virus, Tobacco Mosaic Virus, Beet Yellow Virus, and Turnip Yellow Virus, Etc.

Bacteriophage -The virus which infects bacterial cells is known as Bacteriophage. There are many varieties of bacteriophages, such as DNA virus, RNA virus, λ page, T2,T4 etc.

Insect Virus -The virus which infects insects is known as Insect virus. These viruses are used as a biocontrol agent in agriculture. Ascovirus virions and Entomopox virus are best examples for insect virus.

Viral Reproduction

Lytic cycle = reproduction occurs, kills the host cells.

Lysogenic cycle = virus nucleic acid integrates into host DNA and replicates along with host DNA.

1. **Attachment** - the virus attaches to the Bacteria surface.
2. **Penetration** -the DNA or RNA of the virus enters the cell
3. **Biosynthe**sis - viral components such as viral proteins, viral nucleic acid are made .
4. **Maturation** - assembly of viral Nucleic acids and proteins to make complete virus particle.
5. **Release** - viruses kills and lysis the host cell and new viruses are released out.

Unit - 4: Eukaryotic microorganisms

Q. WRITE NOTES ON FUNGI (8 Marks)

Fungi are eukaryotic organisms that include microorganisms such as yeasts, moulds and mushrooms. The science dealing with the study of fungi is called as mycology.

Occurrence of Fungi:
Fungi occur in air, water soil and on plants and animals. They grow in warm and humid places. Most of the fungi grow in soil, on dead and decaying organic material. They can grow on foods like jam, bread, fruits etc.

MORPHOLOGY:
Fungi exists in two forms, filamentous or hyphal form (MOLD) and singe celled form (YEAST).
Yeast is Unicellular while Mold is multicellular and filamentous
STRUCTURE

- It has a Eukaryotic cell structure. It has cell wall, cell membrane, cytoplasm, cell organelles and nuclei.
- The nucleus is surrounded by a nuclear membrane
- Fungi consist of long thread-like structures known as hyphae. These hyphae together form a structure called mycelium.
- Fungi possess a cell wall which is made up of chitin and polysaccharides.
- Fungi lacks Chloroplast.

NUTRITION

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

- Saprophytic – The fungi obtain their nutrition from dead organic substances. Examples: Rhizopus, Penicillium and Aspergillus.
- Parasitic – The fungi obtain their nutrition from other organisms (plants or animals) and absorb nutrients from their host. Examples: Taphrina and Puccinia.
- Symbiotic – These fungi live in interdependent relationship association with other species in which both are mutually benefited.
  Examples: Lichens and mycorrhiza.

OTHER CHARACTERS

1. Fungi grow best in acidic environment (acidic pH).
2. Fungi can tolerate high sugar concentration and dry condition

3. Most of the fungi are Obligate aerobes (molds) and few are facultative anaerobes (yeasts)
4. Optimum temperature of growth for most saprophytic fungi is 20-30 C while (30-37) C for parasitic fungi.
5. Growth rate of fungi is slower than that of bacteria.
6. Cell wall is composed of chitin
7. Cell membrane consists of ergosterol

REPRODUCTION

Reproduction in fungi is both by sexual and asexual means.

- Vegetative reproduction – By budding, fission and fragmentation
- Asexual reproduction
- Sexual reproduction

Classification of fungi:

The kingdom fungi or mycota is classified into 9 division however only four division are involved in medical mycology

1. Ascomycetes
2. Basidiomycetes
3. Zygomycetes
4. Deuteromycetes

IMPORTANCE OF FUNGI

1. Recycling – They play a major role in recycling the dead and decayed matter.
2. Food – Mushrooms are used as food by humans.
3. Medicines – fungi produce antibiotics which are used to control diseases in humans and animals. Penicillin antibiotic is derived from Penicillium.
4. Biocontrol Agents – Fungi are used as biocontrol agents to control insects, other small worms and help in controlling pests. Spores of fungi are used as spray-on crops.
5. Food spoilage – Fungi are responsible for major spoilage of stored food.
6. fungi are Used in bioremediation (reduces toxic concentration)
7. fungi are Used in agriculture, horticulture and forestry, example; they are used as biofertilizer and biopesticides
8. they are used in biodegradation and bio-deterioration
9. they are Used in industrial fermentation process.

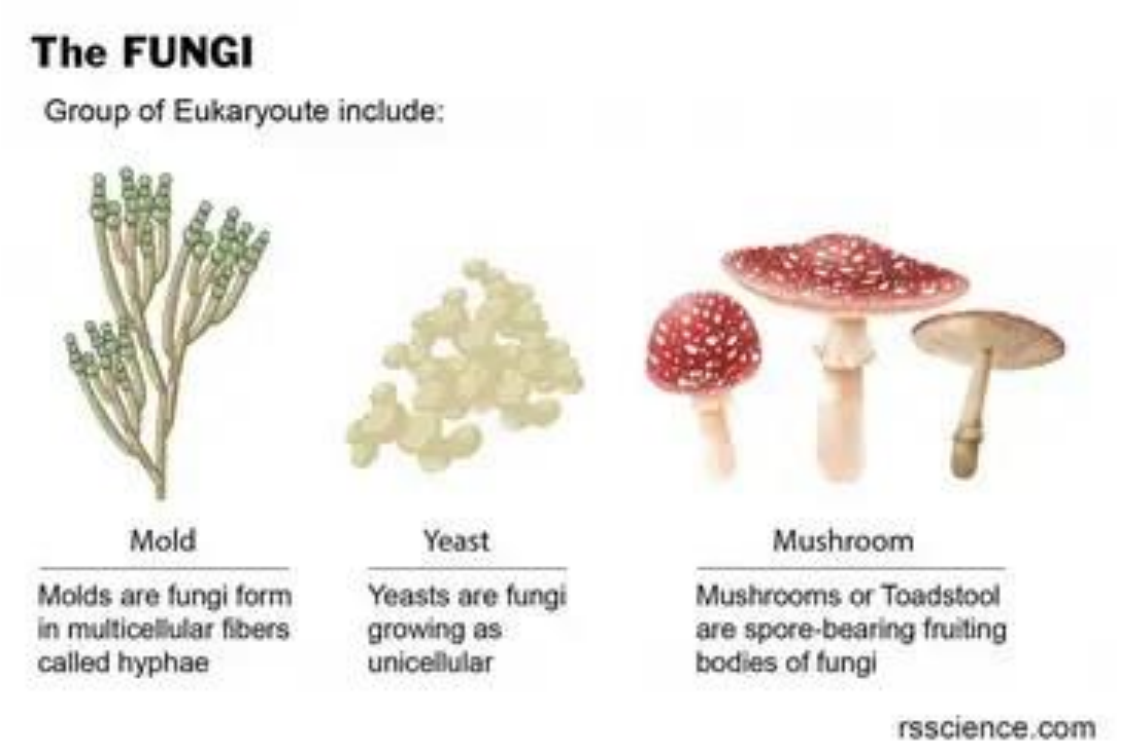
Examples

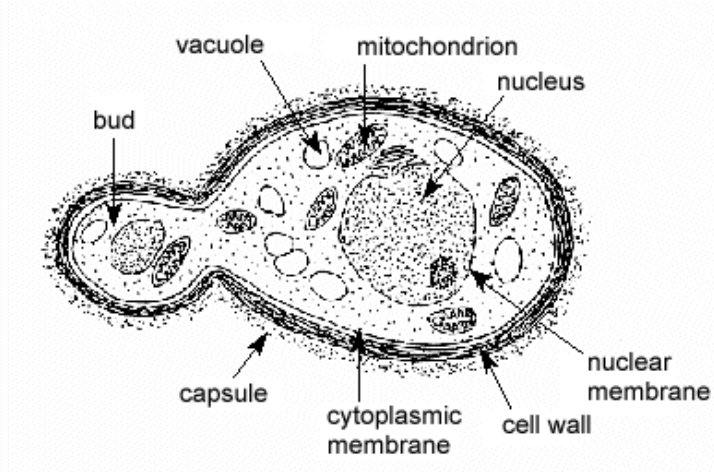
- Penicillium notatum is used for production of penicillin antibiotics
- Aspergillus niger is used for prodution of citric acid
- Saccharomyces cerevisiae is used for alcohol production

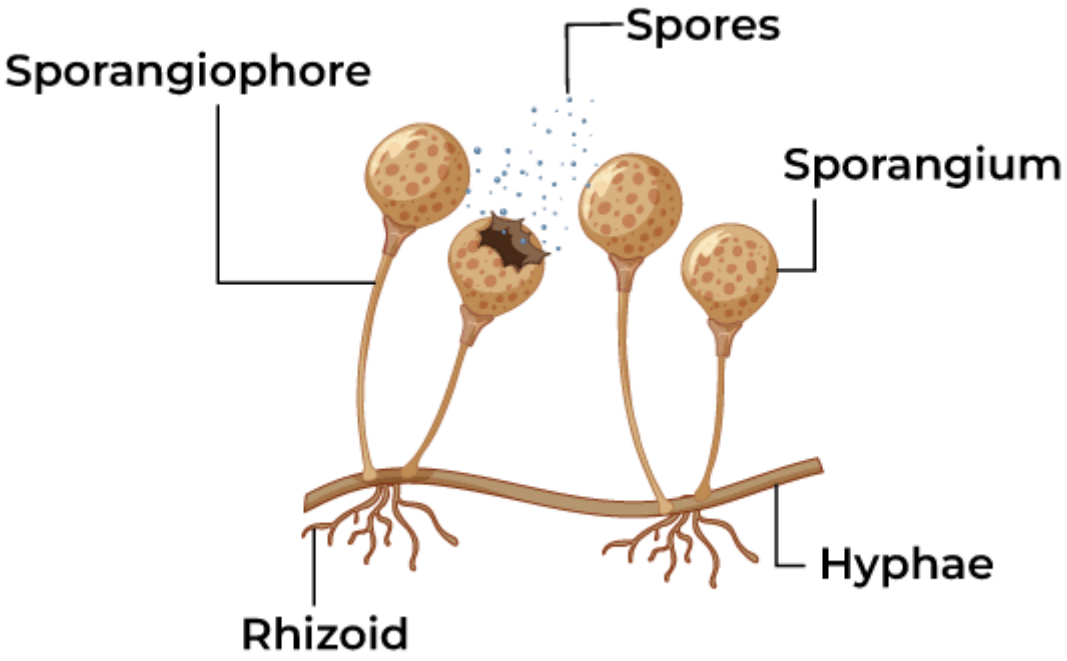
Examples of Fungi

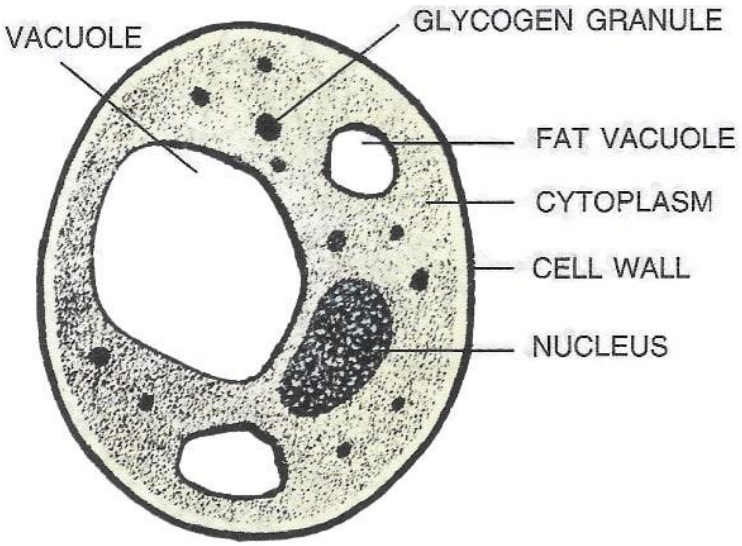
- Yeast- Saccharomyces cerevisiae, Candida, Cryptococcus, Trichophyton, Epidermophyton, Histoplasma

- Mushrooms- Amanita, Agaricus
- Molds- Rhizopus, Mucor, Penicillium notatum, Aspergillus niger






Spores
Sporangiophore
Sporangium
Hyphae
Rhizoid


VACUOLE
GLYCOGEN GRANULE
FAT VACUOLE
CYTOPLASM
CELL WALL
NUCLEUS

Q. WRITE NOTES ON ALGAE (8 Marks)

Algae are green coloured microorganisms found mostly in water. They contain chlorophyll and are photosynthetic. They have simple body structure.

GENERAL CHARACTERSTICS

Algae are found in both fresh and marine water.
Algae are found in many shapes like filamentous, colonial.
Cell wall is made up of cellulose and pectin.
Algae are the simplest multicellular plants. Some are unicellular eg. Chlamydomonas
Algae body is known as Thallus and they are a vascular.
Algae are eukaryotic .
Algae are photoautotrophs.
Storage form of food in algae is Starch
Reproduction: Algae reproduce either by vegetative, asexual or sexual method

REPRODUCTION

1.Vegetative

- Binary fission -Cell is divided into two parts and nucleus is also divided into two parts by mitosis. eg. Found only in unicellular algae.
- Fragmentation-Filaments break down into small pieces & form new filaments. eg.
- Asexual

2.Asexual reproduction- It is a method of reproduction in all unfavourable conditions.

3.Sexual reproduction is of three types

- Isogamous
- Anisogamous
- Oogamous

CLASSIFICATION OF ALGAE -On the basis of photosynthetic pigments algae classified into three classes.

1. Chlorophyceae (Green Algae)
2. Phaeophyceae (Brown Algae)
3. Rhodophyceae (Red Algae).

1. Chlorophyceae (Green Algae)

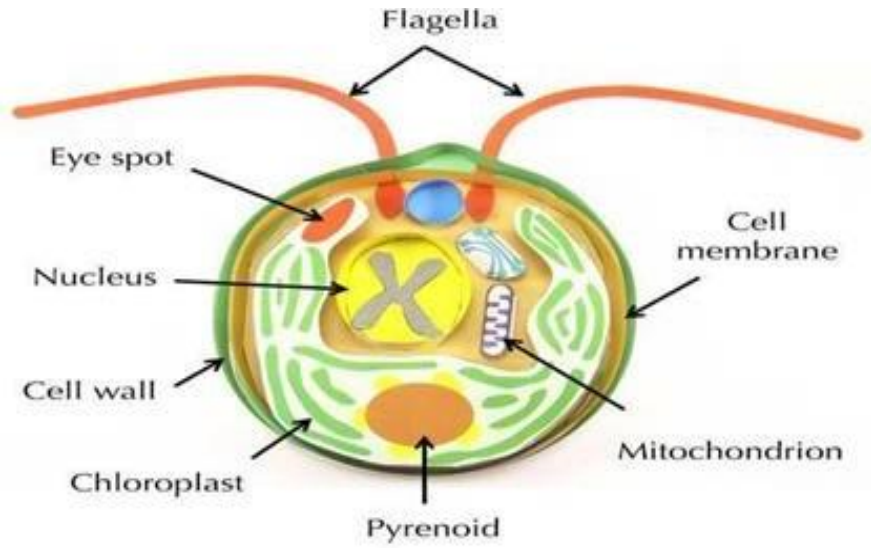
- It is the largest class of algae
- They are commonly known as green Algae.
- Photosynthetic pigments: They possesses chlorophyll a, chlorophyll b and small amount of β-carotenoids.
- Habitat: Mostly freshwater
- They are unicellular as well as multicellular.
- Each cell is eukaryotic
- Thalllus: their body structure, shape and size varies.
- Storage form of food: Starch
- Pyrenoids stores starch
- Cell wall has two layer: outer layer composed of pectose and inner layer is composed of cellulose
- Reproduction: vegetative, asexual and sexual method

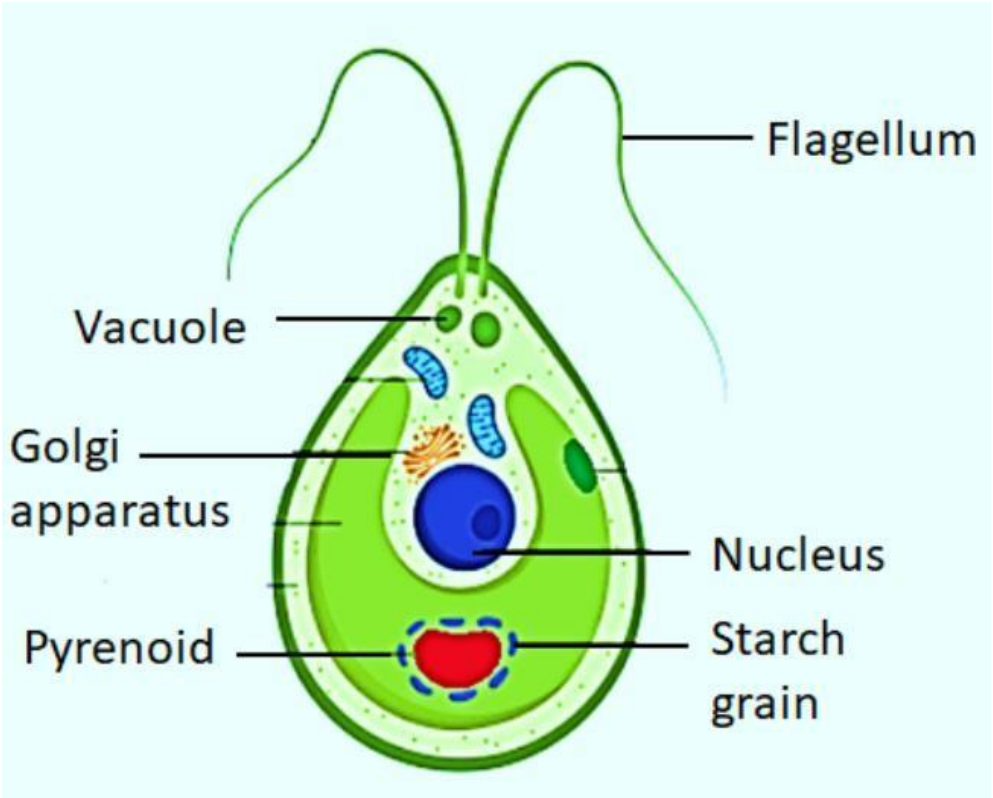
2. Phaeophyceae (Brown algae)

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

3. Rhodophyceae (Red algae)

- Rhodophyceae are commonly known as Red Algae
- Photosynthetic pigments: They possesses Red colored photosynthetic pigments r-phycocyanin and r-phycoerythrin along with chlorophyll a, d, xanthophyll and β-carotenoid
- Habitat: They are aquatic, mostly marine. Some are freshwater
- Thallus: Red algae show a variety of life forms
- Storage form of food: Floridean starch and floridosides sugar.
- Reproduction: vegetative, asexual and sexual mode


Flagella
Eye spot
Cell membrane
Nucleus
Cell wall
Mitochondrion
Chloroplast
Pyrenoid


Flagellum
Vacuole
Golgi apparatus
Nucleus
Pyrenoid
Starch grain

Q. WRITE NOTES ON PROTOZOA (8 Marks)

- Protozoa are unicellular Eukaryotes with membrane bound nucleus and cell organelles. Protozoans are the simple and primitive organisms.

- They are free living or parasitic.
- They are Found in kingdom Protista.
- They are Heterotrophs that ingest small food particles & digest it inside food vacuoles containing digestive enzymes.
- They are Classified by the way they move (cilia, flagella, pseudopodia…). They are Microscopic in size
- They are Found in freshwater, marine, and moist terrestrial habitats.
- Make up part of the zooplankton & serve as food for animals in marine & freshwater systems
- First seen by Leeuwenhoek in 1675
- Many species are free living. Some species are parasitic living in the bloodstream of their host & cause malaria, amebic dysentery, or giardiasis
- The branch of study of protozons is known as Protozoology.

Locomotion is by means of-

(a) Finger-like Pseudopodia e.g. Amoeba

(b) Whip like Flagella e.g. Euglena
(c) Hairy cillia e.g. Paramoecium

(d) By contraction

Nutrition of Protozoans are mainly holozoic (Amoeba), Mixotrophic. (Euglena), Parasitic, Saprozoic (Plasmodium) and Digestion is intracellular take, place in food vacuole. Digestion is intracellular

Respiration and Excretion take place by exchange of gases through body surface. Some excretion may occure through contractile vacuole. Nitrogenous waste is Ammonia. Some fresh water protozoans get rid of excess water through contractile vacuole known as Osmoregulation.

Reproduction occurs by asexual & sexual methods.

Asexual:

(a) Binary fission (Amoeba)

(b) Transverse fission (Paramoecium)

(c) Longitudinal fission (Trypansoma, Euglena)

(d) Multiple fission (Plasmodium)

(e) Budding

Sexual:

(a) Syngamy (Plasmodium), Conjugation (Paramoecium) also form cyst which help in unfavourable condition for reproduction of organism.

Classification of Protozoa

Class 1. Flagellata or Mastigophora

(1) The body is covered by a thin pellicle or cuticle.

(2) The locomotory organs are flagella.

(3) The contractile vacuoles are present in fresh water forms with accessory vacuoles.

(4) Chloroplasts are found in some forms.

(5) They may be free living or parasitic.

Examples: Chrysamoeba, Cryptomonas, Euglena, Volvox, Chlamydomonas,

Class 2. Rhizopoda

(1) There is no definite cell wall or pellicle

(2) There is no definite shape

(3) The locomotory organs are pseudopodia

(5) The contractile vacuoles are present in the fresh water forms.

Examples: Amoeba, E. histolytica, etc.

Class 3. Ciliophora

(1) The body is covered by thin pellicle

(2) They have a fixed permanent shape

(3) The locomotory organs are cilia

(4) Tentacles are present

(5) The class ciliophora is divided into two sub-classes, namely Ciliata and Suctoria.

Class 4. Sporozoa

(1) They are exclusively endoparasitic

(2) The body is covered by pellicle.

(3) Reproduction takes place by spore formation

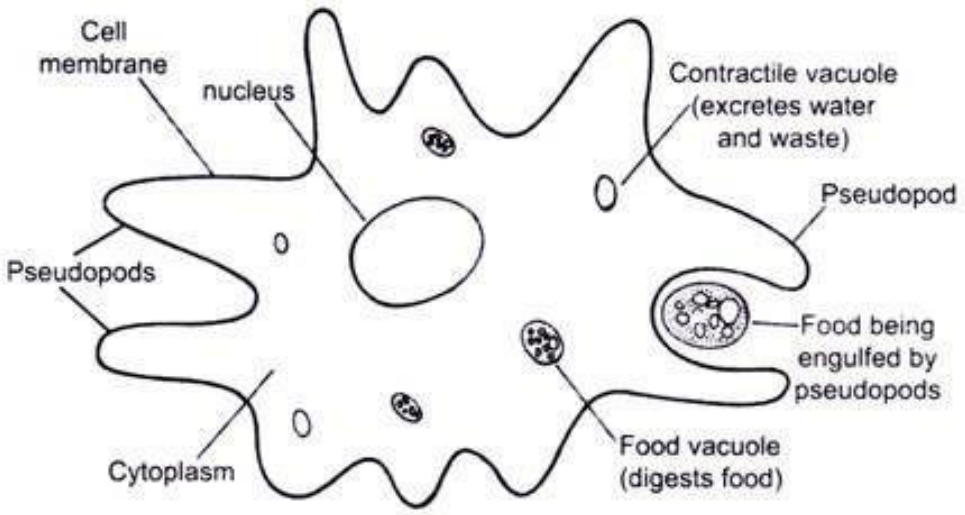


Fig. 9.7 *Amoeba proteus*

Unit - 5: Growing Microbes in Lab: Five I's

Q. DESCRIBE INOCULATION METHODS (4 Marks)

Inoculation in microbiology is the process of introducing microbes into a culture media, allowing them to reproduce.

Following methods are used -

1. Inoculating Loops/Inoculating Needles
2. Spread Plate
3. Streak Plate
4. Pour Plate
5. Stab Culture
6. Slant Culture
7. Liquid Culture

1. INOCULATING LOOPS AND INOCULATING NEEDLES

Inoculation loops or inoculation Needle are tools used by microbiologists to transfer inoculum (a small sample of microbe culture). The tool comprises a slender handle with a small loop at the end.

2. SPREAD PLATE TECHNIQUE

The spread plate technique involves using a sterilized spreader L-shaped rod. A small amount of bacterial culture usually 0.1mL is applied on a sterile agar plate and spread evenly all over the plate using L-Shaped glass rod. The plates are incubated, and the colonies are counted.

3. STREAK PLATE METHOD

Streak literally means long thin line. In this method a sample is spread in a petri dish in the form of a long thin line over the surface of solid media.
The purpose of the streak plate method is to produce an isolated colony of an organism on the agar plate. Streaking method was first developed by Loeffler and Gaffky in Koch's laboratory. It involves the dilution of bacteria by streaking them over the agar in a Petri dish to obtain isolated colonies.

4. POUR-PLATE TECHNIQUE

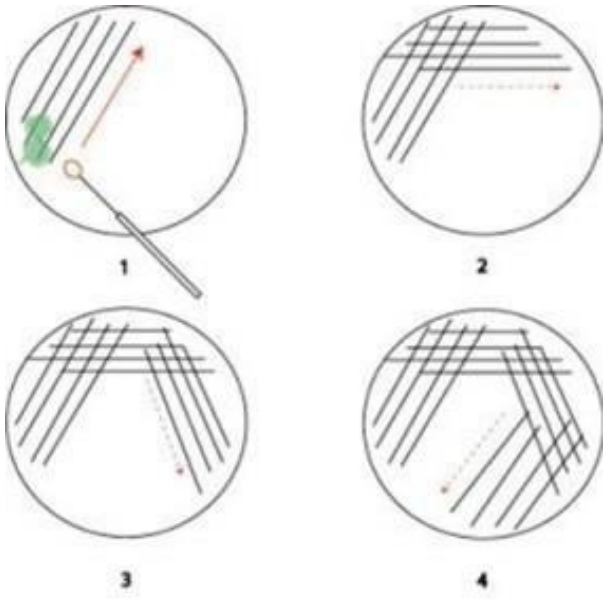
The purpose of the pour plate method is to produce isolated colonies on the agar plate. It requires sample to be in suspension. The sample is added to the petri dish and then molten agar is poured over it . In other methods the sample is

mixed with the molten agar medium and then poured into empty sterile petri dishes.

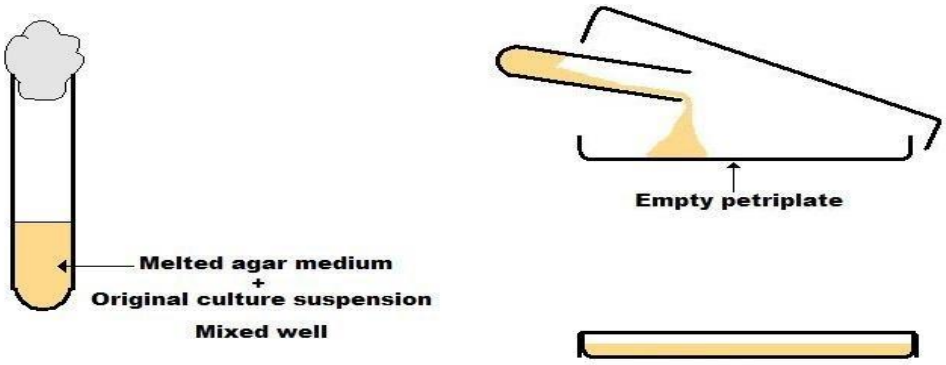
5. STAB CULTURES: Stab cultures are made by pouring the agar medium into a test tube and solidifying it. Bacteria are introduced through an inoculation needle . Bacteria grow in the punctured area. Stab cultures are most commonly used for short-term storage or shipment of cultures.

6. AGAR SLANT- An agar slant tube (or simply an agar slant) is a screw-capped culture tube partly filled with an agar mix such as nutrient agar or TSA . To make it a slant tube the agar is allowed to cool with the tube laying at an angle, resulting in a large surface area for spreading a culture.

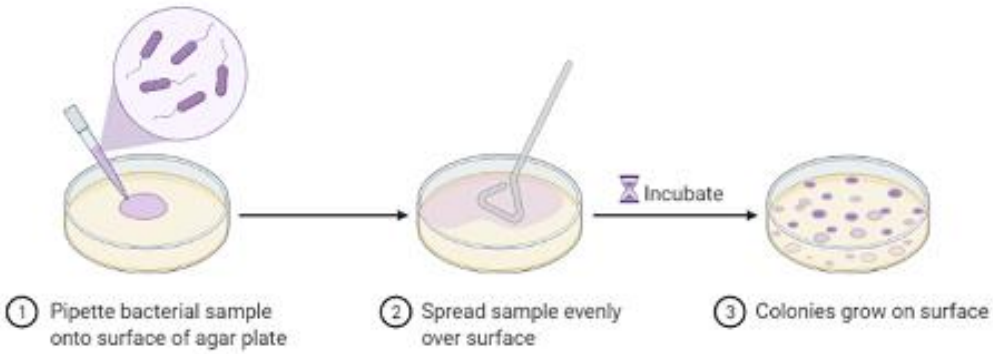
7. LIQUID CULTURES- Broth cultures or s liquid cultures used to grow bacteria in laboratories. A sterile liquid growth medium such as nutrient broth is inoculated with bacteria and placed in an incubator at the appropriate temperature.



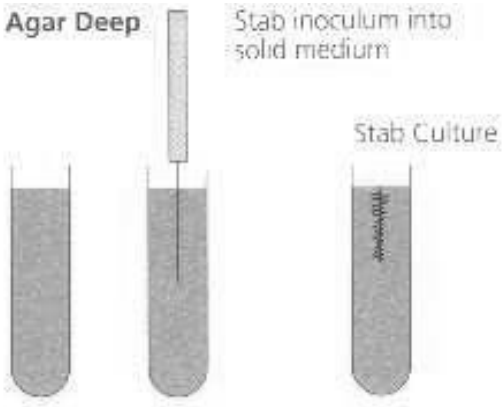
STREAK PLATE



POUR PLATE



SPREAD PLATE



STAB CULTURE

Q. NUTRIENT BROTH & NUTRIENT AGAR (4 Marks)

Nutrient Broth is a liquid medium used for the cultivation of microbes. It supports the growth of a wide range of microorganisms. It consists of peptone, beef extract NaCl. This medium provides the nutrients necessary for the growth of a large number of microorganisms.

Composition of Nutrient Broth

Beef Extract .3.0 g
Petone .5.0 g
Nacl .5.0g
Distilled Water……..……100 ml
pH – 7

Composition of Nutrient Broth

- Peptone- Peptone is the principal source of organic nitrogen for the growing bacteria.
- Beef extract/yeast extract- It contains carbohydrates, organic nitrogen compounds and vitamins and salts.
- NaCl-*it* maintains a salt concentration in the medium that is similar to the cytoplasm of the microorganisms.
- Distilled water
- pH is adjusted to neutral *(7.4) at 25 °C.*

Preparation of Nutrient Broth

- Add .5 g peptone, .3 g beef extract,. 5g NaCl to 100 ml distil water. Adjust the ph to 7.
- Heat this mixture while stirring to fully dissolve all components.
- Autoclave the dissolved mixture at 121 degrees Celsius for 15 minutes.
- After autoclaving nutrient broth allow it to cool & inoculate with Microorganisms.

Uses of Nutrients Broth

- It is used for multiplication of microbes.
- It is used for the cultivation of microorganisms in Microbiology lab, industries, agriculture, medicine, pharma companies etc.

NUTRIENT AGAR
Nutrient Agar is a solid medium used for the cultivation of microbes. It supports the growth of a wide range of microorganisms.

Composition of Nutrient Agar

Beef Extract	.3.0 g
Petone	.5.0 g
Nacl	.5.0g
Agar	4.0 g
Distilled Water…… …….……100 ml	

Final pH - 7

Nutrient agar contains same ingredients as Nutrient broth except agar.

Uses of Nutrients Agar

- It is used for isolation and purification of bacterial cultures.
- Nutrient Agar/broth is used for the cultivation and maintenance of microorganisms .
- It is also used for enumeration of organisms in water, sewage, dairy products.

Q. PURE CULTURE TECHNIQUE (4 Marks)

Pure culture in microbiology contains a single species of organisms. To obtain a pure culture the following are used -

- **Spread Plate**
- **Streak Plate**
- **Pour Plate**

2. SPREAD PLATE TECHNIQUE

Spread plate technique is the method of isolation of microorganisms. It is easy simple and economical method.The spread plate technique involves using a sterilized spreader L-shaped rod. A small amount of bacterial culture usually 0.1mL is applied on a sterile agar plate and spread evenly all over the plate using L-Shaped glass rod. The plates are incubated, and the colonies are counted. This procedure requires

- Bacteria Culture
- Sterile Nutrient Agar Plate
- Inoculation Loop
- L-Shaped Glass Rod
- Bunsen Burner
- Alcohol

3. STREAK PLATE METHOD

Streak literally means long thin line. In this method a sample is spread in a petri dish in the form of a long thin line over the surface of solid media.
The purpose of the streak plate method is to produce isolated colonies on the agar plate. Streaking method was first developed by Loeffler and Gaffky in Koch's laboratory. It involves the dilution of bacteria by streaking them over the agar in a Petri dish to obtain isolated colonies.
This method requires the following-

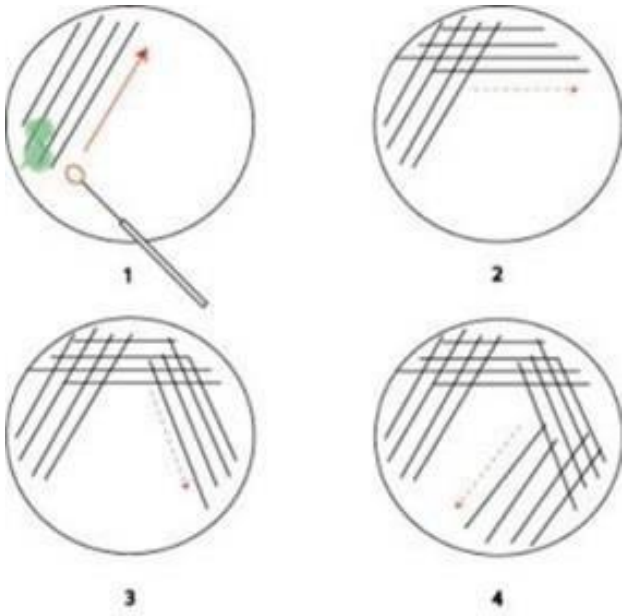
- Bacteria Culture
- Sterile Agar Plate
- Inoculation Loop
- Bunsen Burner

4. POUR-PLATE TECHNIQUE

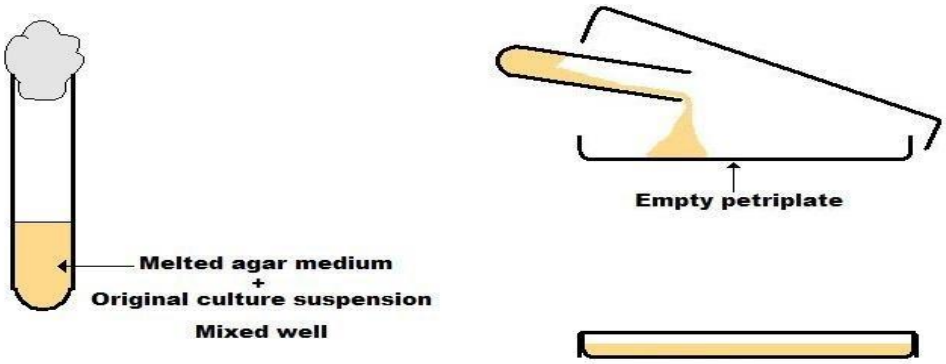
The purpose of the pour plate method is to produce isolated colonies of microorganism on the agar plate. It is simple and easy.It requires sample to be in suspension. The sample is added to the petri dish and then molten agar is

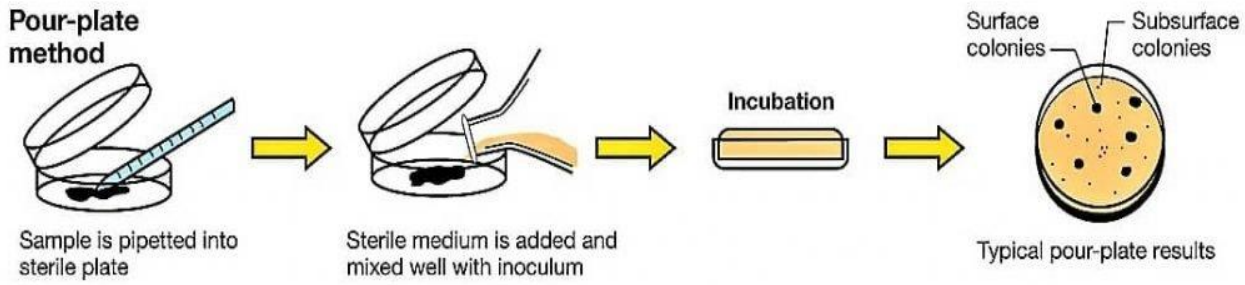
poured over it . In other methods the sample is mixed with the molten agar medium and then poured into empty sterile petri dishes.
. This procedure requires

- Bacteria Culture
- Molten agar at 45 degrees Celsius in a water bath
- Sterile empty Petri Plates
- Pipette
- Test Tubes

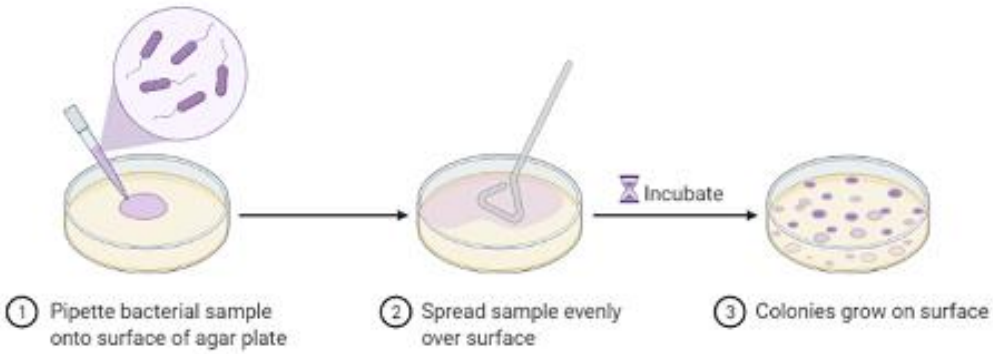


STREAK PLATE





POUR PLATE



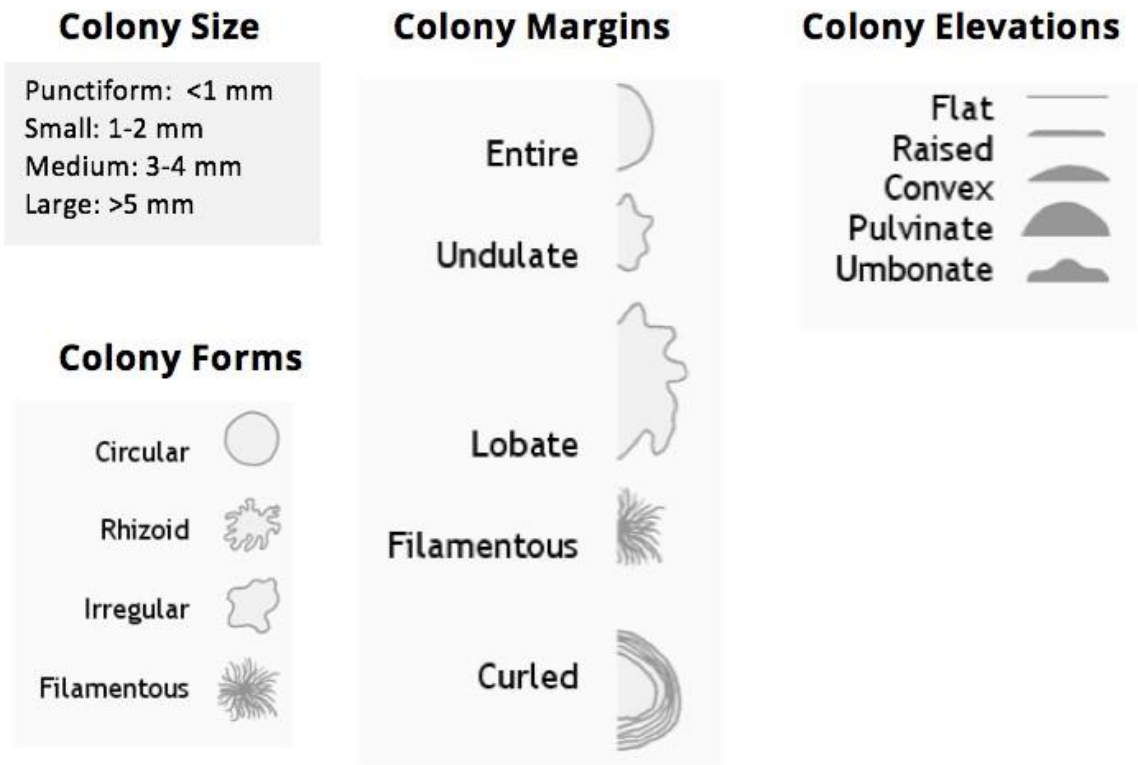
SPREAD PLATE

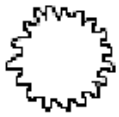
Q. COLONY CHARACTERS OF BACTERIA (4 Marks)

A bacterial colony is a group of bacteria derived from the same mother cell. A single mother cell reproduces to make a group of genetically identical cells, and this group of cells form a colony.

Some Characteristics of a colony are shape, edge, elevation, color and texture,, optical properties such as translucence, sheen and texture (moist, mucoid or dry).

- Form –basic shape of the colony. For example, circular, filamentous, etc.
- Size – the diameter of the colony. Tiny colonies are referred to as punctiform.
- Elevation – this describes the side view of a colony. Turn the Petri dish on end.
- Margin/border – the edge of a colony.
- Surface – how does the surface of the colony appear. For example, smooth, glistening, rough, wrinkled or dull.
- Opacity – for example, transparent (clear), opaque, translucent (like looking through frosted glass), etc.
- Colour (pigmentation) – for example, white, buff, red, purple, etc.



MARGIN	COLOUR	ELEVATION	TEXTURE	SHAPE
 Curled	 Orange	 Raised	Slimy, moist	 Round
 Entire (smooth)	 Red or pink	 Umbonate	Matte, brittle	 Punctiform
 Filamentous	 Black	Flat	Shiny, viscous	 Rhizoid (root-like)
 Undulate (wavy)	 Brown	 Convex	Dry, mucoid	 Filamentous
 Lobate	 Opaque or white	 Pulvinate (Cushion-shaped)	Translucent	 Irregular
 **Erose (serrated)**	**Milky**	**Growth into culture medium**	**Iridescent (changes colour in reflected light)**	 **Spindle**

Q. WET MOUNT SLIDE (4 Marks)

A wet mount is made by placing a fluid solution on a slide, suspending a specimen in a solution, and then covering the specimen and the solution with a cover slide.

DIFFERENT TYPES OF WET MOUNTS

Wet mounts can be made using several different kinds of liquids. Water, immersion oil and glycerin (glycerol) can be used. Water is most commonly used.

Example of Wet mount- pond water, cheek cells, blood or sperm samples.

Materials Required:

- Microscopic slides
- Coverslips
- Inoculating loop
- Micropipettes
- Bunsen burner
- Sample

Procedure:

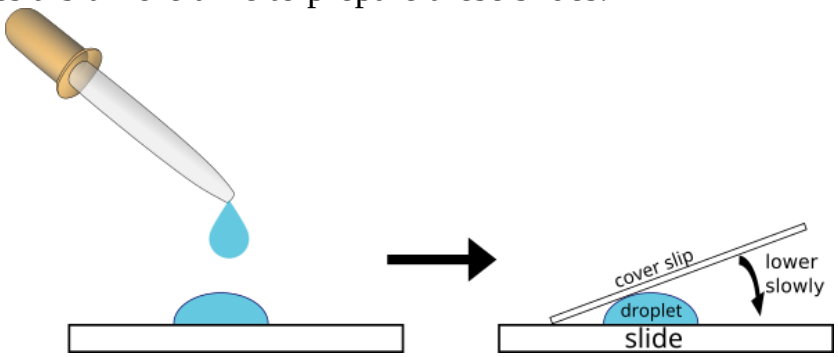
1. Take a clean, scratch free glass slide.
2. Label the slide with the name of the organism
3. Place 15 - 20 uL of the culture in the middle of the slide
4. Lower a clean cover slip over the drop as though it were hinged at one side avoiding bubbles
5. Examine the preparation under microscope first under 4 x followed by 40 x and 100x magnification
6. Identify the organisms
7. Clean up the slide with alcohol first (because it had live bacteria on it), followed by soap and water.
8. Discard the cover slip.

ADVANTAGES –

- This type of slide preparation allows you to view microscopic living things without them drying out.
- It easily helps in identifying the motility of the microorganisms.

DISADVANTAGES –

- It dries within few minutes, making it difficult to visualise or identify the live organism.
- It takes a bit more time to prepare these slides.



Q. SIMPLE STAINING (4 Marks)

Simple staining is one of the common staining techniques. It is a very simple or **direct staining method** that uses a **single stain** only. The microorganisms are invisible to the naked eye, and to make them visible, staining is performed.
Direct staining makes the use of basic dyes like -

- Methylene blue
- Safranin
- Crystal violet
- Malachite green

The simple stains make the organism visible and help us examine the organism's shape, size, and arrangement.

Procedure of Simple Staining - It involves the following three steps:

1. Smear preparation
2. Heat fixing
3. Staining

Smear Preparation

A bacterial smear is a thin film of bacterial culture. For the smear preparation, we need to perform the following steps:

- Take a clean, grease-free glass slide.
- Add a drop of distilled water to the centre of the glass slide.
- Then, add inoculum from the bacterial culture.
- mix the inoculum with the inoculating loop until a thin bacterial film is formed.

Heat Fixing

After smear preparation the prepared slides are run over the Bunsen burner's flame at least three times. Then, allow the slide to air dry.
Heat fixing helps in the fixation of a specimen to the glass slide.
Heat fixing helps the stain to penetrate the smear.

Staining of Bacteria

It is the last step. This stage involves the following steps:

- Add stain to the heat fixed smear.
- Allow the stain to stand for at least 1 minute.
- Wash off the glass slide carefully.
- Blot dry the slide with absorbent paper (do not wipe the slide).
- Examine the glass slide under the microscope

Advantages

- It is a very **simple** method. It stains the organism by using a single reagent.

- It is a **rapid** method and takes only 3-5 minutes.
- It helps to examine the bacterial shape, size and arrangement.
- It also helps us to differentiate the bacterial cells from the non-living structures.

Disadvantages

- It does not give much information about other characteristics of bacteria.