MICROBIOLOGY

UNIT 1 NOTES

INTRODUCTION TO MICROBIOLOGY

- HISTORY OF MICROBIOLOGY
- PRAKARYOTES AND EUKARYOTES
- ULTRASTRUCTURE OF BACTERIA
- MORPHOLOGICAL CLASSIFICATION
- NUTRITIONAL REQUIREMENTS
- CULTIVATION OF ANAEROBES
- MICROSCOPY

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MICROBIOLOGY

- The term 'Microbiology' was first introduced by Louis Pasteur.
- Microbiology is simply defined as study of Microorganism / Microbes.
- Microorganism are those living things that are too small to be seen with naked eyes, they can only be seen by using a Microscope.
- The diverse group of microorganism includes Algae, Archae, Bacteria Fungi, Protozoa, Viruses etc.

HISTORY OF MICROBIOLOGY

The History of Microbiology can be studied in 4 different Era.

① Discovery Era
② Transition Era
③ Golden Era
④ Modern Era

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① DISCOVERY ERA

- It is also known as Spontaneous Era.
- The Era starts with Aristotle (384 - 322 BC) who believed that
- Living Organism could developed from Non- Living things.

Robert Hooke (1635 - 1703)

In 1665, Robert Hooke discovered smallest unit of tissues, which he called 'Cells' & published his work in his book Micrographia.

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Antonie Van Leeuwenhoek (1632-1723)

- He was a Dutch Microbiologist and known as Father of Microbiology.
- He was the first person who observed & accurately describe Microorganism which he named Animalcules (little animals) by using his self made Microscope.
- Actually he was a dutch linear Merchant but spent much of his time constructing microscope & studying about Microorganism.

② TRANSITION ERA

Development of Microbiology raised some important questions towards the belief of Spontaneous Generation. (Theory that says living organism can be developed from Lifeless objects) because at that time theory of spontaneous generation was accepted widely.

Francisko Redi (1626-1697)

- He was the first scientist who challenged the theory of spontaneous generation
- He carried out a series of experiments on decaying meat and its ability to produce maggots spontaneously.
- He shows that maggots would not arise from meat, if it is covered.

John Needham (1713-1781)

- He was actually the supporter of spontaneous theory.
- He proposed that tiny microorganism arose spontaneously on mutton.
- He covered the flask and even heated it still the microbes appears on mutton broth.

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Lazzaro Spallanzani (1729-1799)

- He was an italian scientist, he dispro disapproved all the experiments performed in the favour of spontaneous generation.
- He demonstrated that air carries germs.
- He boiled the mutton broth for longer period, removed the air from the flask and then sealed the container and observed no appearance of microorganism.
- He disproved the theory of spontaneous generation and proposed the theory of Biogenesis.
- The theory of biogenesis was later proved and supported by Louis Pasteur.

③ GOLDEN ERA

- The Golden Era of Microbiology was the time period during which science of microbiology bloossomed.
- The Era starts with the work of Louis Pasteur & Robert Koch who had their own research institute.
- During this period, we see the real begining of Microbiology.

Louis Pasteur (1822-1895)

- He was the first scientist who introduced the word 'Microbiology' for the first time and officially known as 'Father of ~~Microbiology~~ Modern Microbiology'.
- Louis Pasteur finally put the concept of spontaneous generation to rest & proved the theory of Biogenesis.
- He also proposed the 'Germ Theory of Disease'.
- He is also well known for his theory of 'Pasteurization'.

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Robert koch (1843- 1910)

- He was a German Practitioner, provided remarkable contributions in the field of Microbiology.
- He proved the germ theory of diseases and demonstrated the role of bacteria in causing disease.
- He introduced solid media for the culture of bacteria.
- He also introduced methods for isolation of bacteria in pure culture.
- He introduced the staining techniques by using aniline dye.
- He discovered the bacterias such as anthrax bacilli, tubercle bacilli and chlorella bacilli.

Lord Joseph Lister (1827-1912)

- He is popularly known as 'Father of Antiseptic Surgery'
- Lister concluded that wound infections were too due to microorganism.
- He proposed a method to destroy microorganism in the operation theatre by spraying a fine mist of carbolic acid into the air.

Paul Ehrlich (1854- 1915)

- He was a German scientist and known as 'Father of Chemotherapy'.
- He discovered the treatment of syphilis by using Arsenic

Alexander Flemming (1888 - 1955)

- In 1929, Sir Alexander Flemming discovered the 'Penicillin' the first antibiotic drug
- Penicillin was the first antibiotic & known as wonder drug.

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④ MODERN ERA

- By the 1970s research on bacterial physiology, biochemistry and genetics had advanced to such an extent that it was possible to study the genetic material of living organism very easily.
- The Genetic Code was decoded by Prof Har Gobind Khorana along with Nirenberg & co workers and also get awarded with Nobel Prize for his discovery.
- Microbiology is the evolving field of biological science and many researches in this field are yet in the process.

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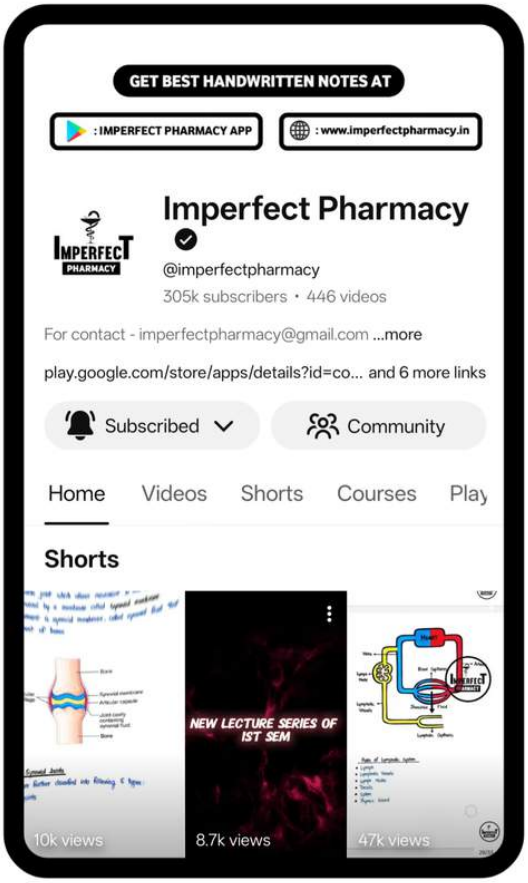

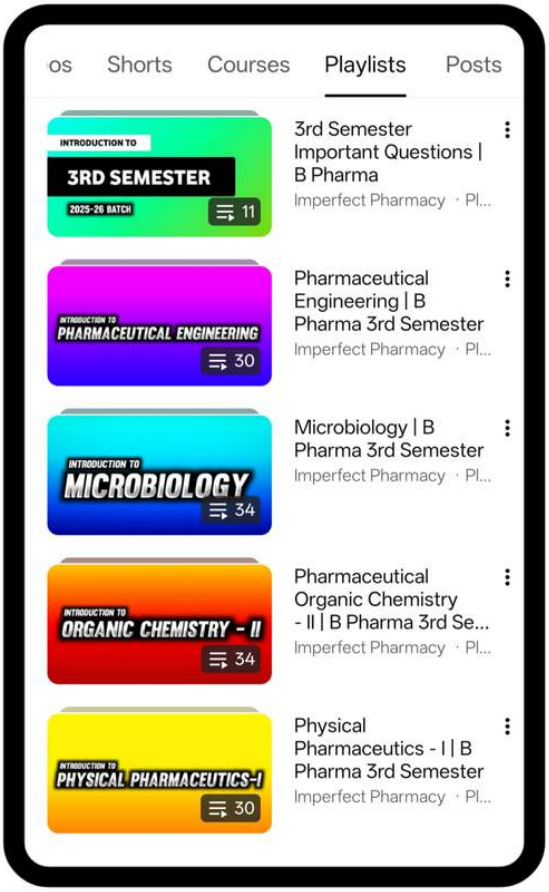

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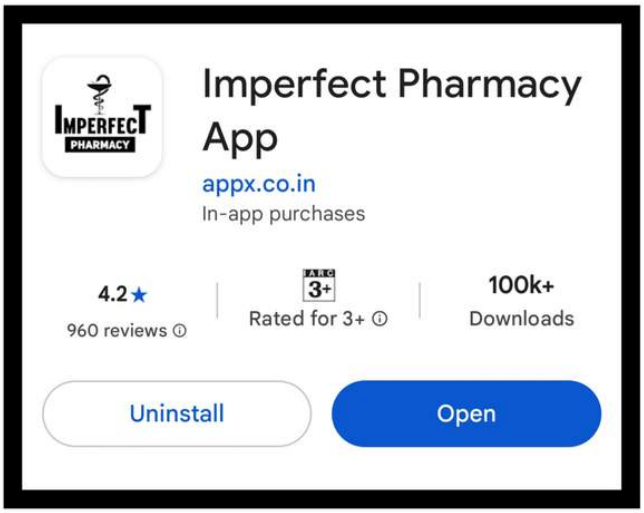
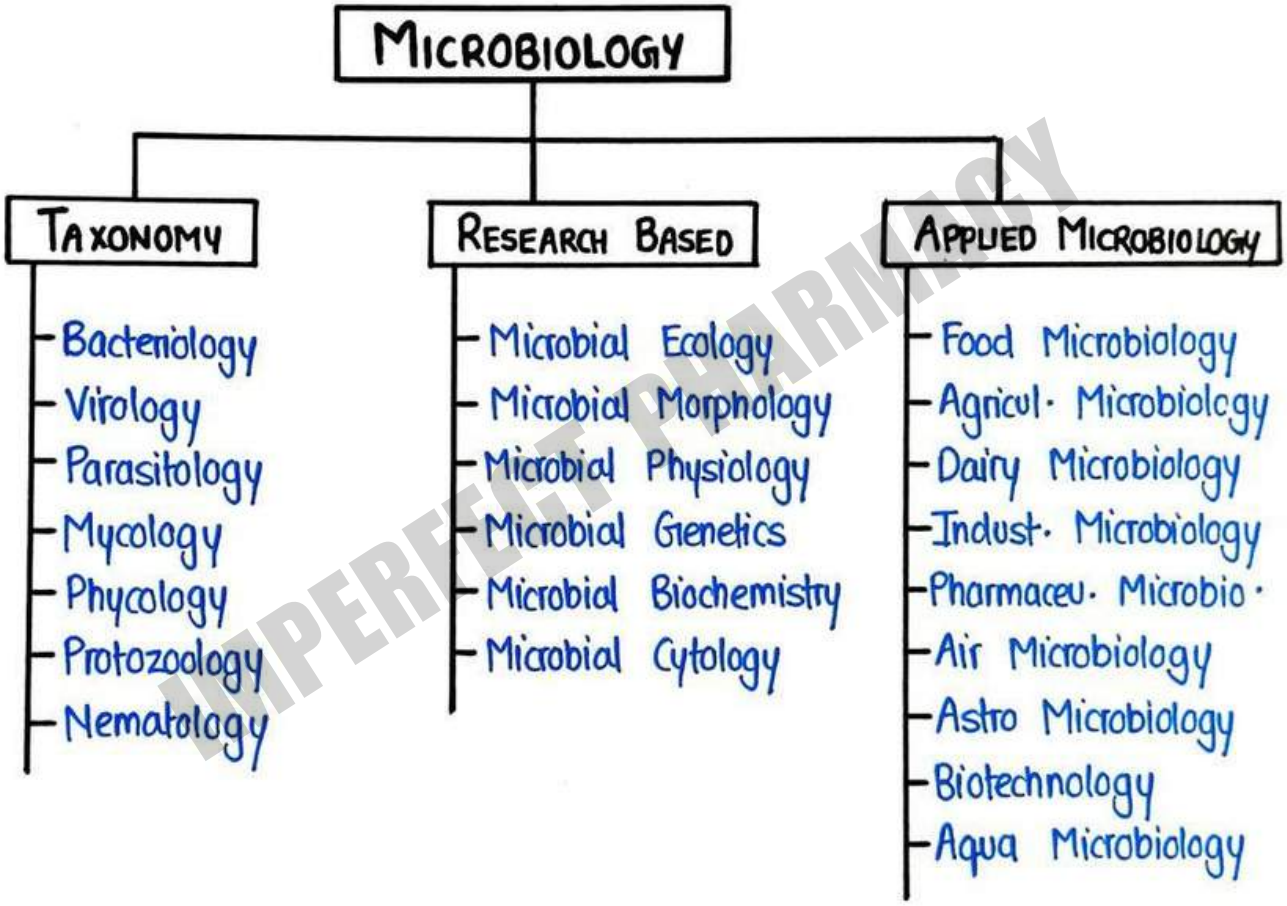


BRANCHES OF MICROBIOLOGY

Microbiology is the largest and most complex branch of biological science, On the basis of taxonomy, research work and the field where it applied, microbiology can be divided into following parts :



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TAXONOMICAL CLASSIFICATION

① **Bacteriology** : Study of Bacteria, Archae

② **Virology** : Study of Viruses & Viral Diseases

③ **Parasitology** : Study of Parasites & Parasitism

④ **Mycology** : Study of Fungi

⑤ **Phycology** : Study of Algae

⑥ **Protozoology** : Study of Protozoa

⑦ **Nematology** : Study of Nematodes

RESEARCH - BASED CLASSIFICATION

① **Microbial Ecology** : It is the study of interrelationship between Microorganism & environment.

② **Microbial Morphology** : Study of detailed structure of Microbes.

③ **Microbial Physiology** : Study of Cellular functions and growth of Microorganism / Microbes.

④ **Microbial Genetics** : It is the study of Genetic Material, Heredity & variation in varieties of Microbes.

⑤ **Microbial Biochemistry** : It is the study of Microbial enzymes & biochemical reactions occuring inside microbes.

⑥ **Microbial Cytology** : Study of Microscopic & submicroscopic detals of Microbes.

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APPLIED MICROBIOLOGY

① **Food Microbiology** : Study of microorganism causing food spoilage & food born diseases & also the use of microbes for food production.

② **Agricultural Microbiology** : Study of relationship blw Microbes & crops
It can be further classified into :
Plant Microbiology & Soil Microbiology.

③ **Diary Microbiology** : Study of Microorganism that are associated with milk & milk products.

④ **Industrial Microbiology** : Study of microbes to use in industrial process to create product in mass quantities.

⑤ **Pharmaceutical Microbiology** : Study of Microbes that are related to the production of pharmaceutical products such as Antibiotics, Vaccines etc & that cause contamination & spoilage.

⑥ **Air Microbiology** : Study of Microbes that cause disease through Air.

⑦ **Astro Microbiology** : Study of Microorganism in outer space.

⑧ **Biotechnology** : Study of Microorganism related to Genetic Engineering.

⑨ **Aquatic Microbiology** : Study of Microbes & their activity concerned with Human & Animal health that are found in water.

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Scope Of Microbiology

Microbiology has a wide range of scope as follows :

- Production of Antibiotics.
- Production of Vaccines, Enzymes.
- Production of Food & Diary Products.
- In Agriculture & Soil Fertility
- Genetic Engineering
- In Industries
- In biochemistry & Physiology
- Sterilization

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PROKARYOTES

- Prokaryotes are those organism that do not have a nucleus in their cells.
- The cell of Prakaryotes is known as 'Prokaryotic Cells'.
- These cells are tend to be smaller and usually found in Unicellular Organism. Example : Bacteria, Archae etc.

EUKARYOTES

- Eukaryotes are those organism that posses a nucleus in their cells.
- The cell of Eukaryotes is known as 'Eukaryotic Cells'.
- These cells are tend to be larger compare to Prokaryotic cells & usually found in multicellular organism. Example : Fungi, Algae etc.

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DIFFERENCE BETWEEN PROKARYOTIC & EUKARYOTIC CELLS

Prokaryotic Cells	Eukaryotic Cells
• They are smaller in size.	• They are larger in size.
• Nuclear Region (Nucleoid) don't have membrane.	• Nucleus is surrounded by Nuclear Membrane.
• Posses Single Chromosomes.	• More than 1 chromosome.
• Nucleolus is Absent.	• Nucleolus is Present.
• Usually Unicellular	• Usually Multicellular.
• 70s ribosomes	• 80s Ribosomes.

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BACTERIA

- The word bacteria is derived from greek word 'bacterium' that means 'rod' or 'small stick'.
- Bacteria are very small microorganism which are visible only under the microscope.
- These are the most abundant 'Prokaryotes'.
- Most of the bacteria perform positive & vital role in nature but few of them are harmful & cause disease & infections.
- The branch of Microbiology that deals with study of bacteria is known as Bacteriology.

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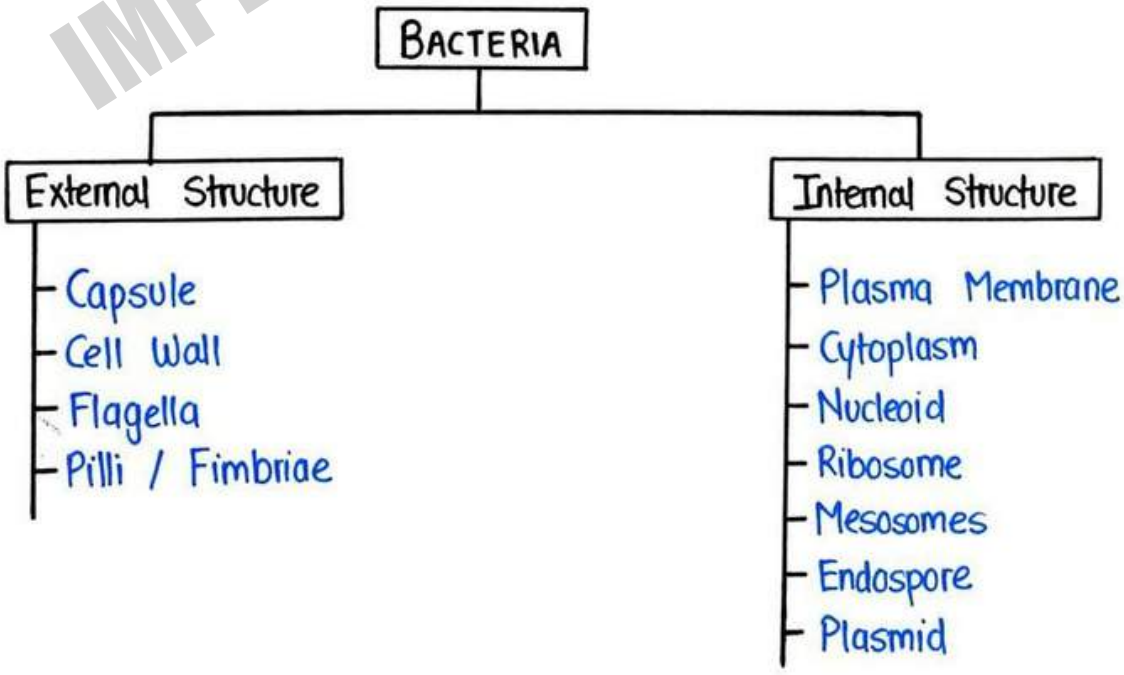
ULTRASTRUCTURE OF BACTERIA

Examination of Bacterial cells with electron microscope reveals various component structures of bacteria, some of them presents outside the bacteria while some present inside the bacteria.



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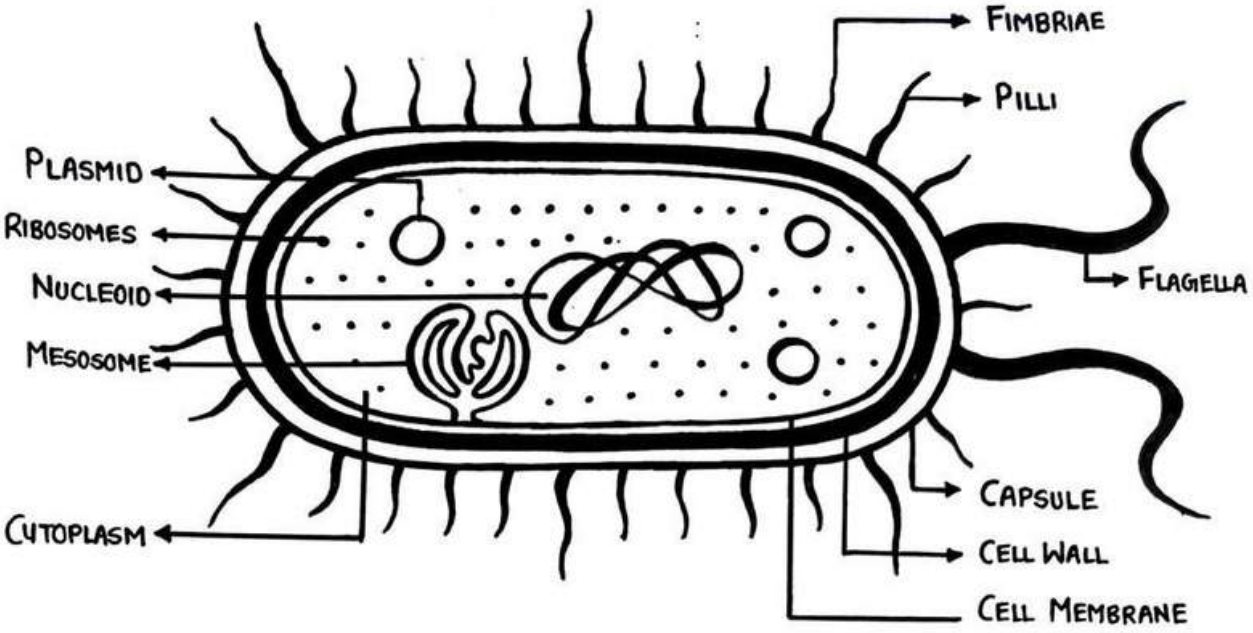

EXTERNAL STRUCTURES

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① FLAGELLA

- Flagell (singular - Flagellum) are long hair like structures .
- They are mainly responsible for locomotion (motility) of bacteria.
- It is further divided into 3 parts : Filament , Hook & Basal Body
- Flagell can be arranged in 5 different types as follows :

(i) Atrichous

(ii) Monotrichous

(iii) Amphitrichous

(iv) Lophotrichous

(v) Peritrichous

② PILLI & FIMBRIAE

- Pili & Fimbriae are short & hollow hair like structures .
- Both Pili & Fimbriae helps to attach bacteria to other surfaces , but Pili are longer & lesser in number compare to Fimbriae.
- Sex Pili helps in conjugation of bacteria.

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③ CAPSULE

- It is the outermost covering of bacteria.
- It is made up of either Polysaccharides or Polypeptides or Both.
- It protects the bacteria from Phagocytosis & antibacterial agents.
- The capsule can be of two types :

(i) Macro - Capsule > 0·2 µm

(ii) Micro - Capsule < 0·2 µm

④ CELL WALL

- It is the middle covering of bacteria lies between capsule and Plasma Membrane.
- It is a rigid structure gives strength & shape to the cell.
- The cell wall of bacteria is made up of Peptidoglycan which is repeating subunit of N-Acetyl Glucosamine (NAG) & N-Acetyl Muramic Acid (NAM).
- Gram Positive Bacteria → Thick Cell Wall.
- Gram Negative Bacteria → Thin Cell Wall

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INTERNAL STRUCTURES

① PLASMA MEMBRANE

- It is the innermost layer / covering of bacteria.
- It can also be known as Cytoplasmic Membrane, Cell Membrane or Inner Membrane.
- It is mainly composed of Phospholipids, Proteins & Carbohydrates.
- It acts as a semi-permeable membrane that allows only selective material to move inside & outside of the cell.

② CYTOPLASM

- It is a colourless, semi-transparent, elastic & thick fluid enclosed within plasma membrane.
- It consist 80% water & remaining 20% includes suspended organic & inorganic solutes.
- It helps in cellular growth, metabolism and reaction.

③ RIBOSOMES

- Bacterial Ribosomes are of 70s type present in cytoplasm.
- Ribosomes are tiny small granular structure.
- It mainly composed of RNA & Proteins.
- The main role of RNA is to synthesize bacterial Proteins & Enzymes.

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④ PLASMID

- Plasmid are small circle of DNA.
- Bacterial cells have many plasmids.
- Plasmids are used to exchange DNA b/w bacterial cells.

⑤ MESOSOMES

- Mesosomes are organelles formed by Plasma Membrane infoldings.
- They are found in the membranes of bacterial cells.
- They serves in DNA replication.

⑥ NUCLEOID

- Instead of Nucleus, bacterial cells contains Nucleoid.
- Nucleoid is an irregularly shaped region within the prokaryotic cell that contains all or most of the genetic materials.

⑦ ENDOSPORE

- An Endospore is a dormant, tough & Non- reproductive structure produced by certain bacteria under stress.
- Endospores are usually produced during unfavorable environmental conditions.
- They are usually occurs in Gram Positive Bacteria.

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MORPHOLOGICAL CLASSIFICATION OF BACTERIA

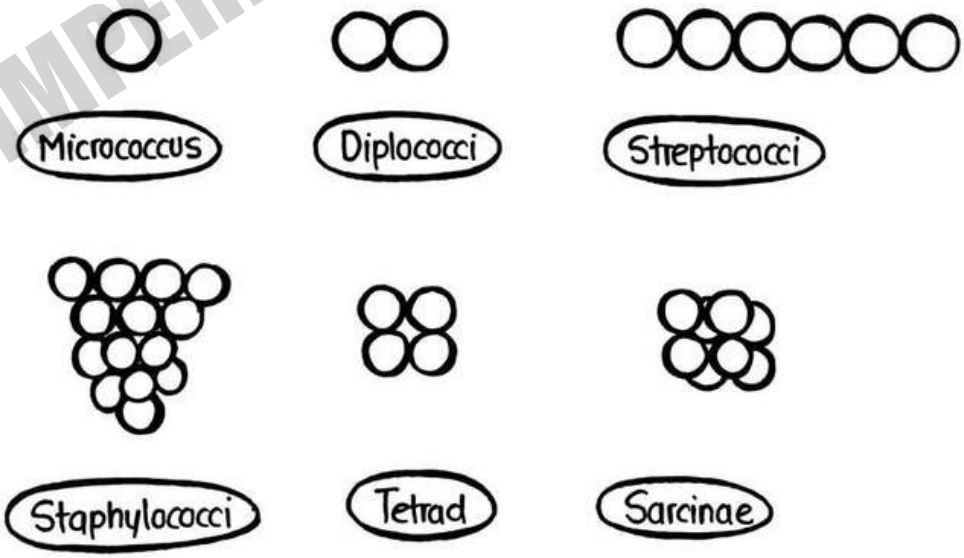
On the basis of Morphology bacteria can be classified according to following 3 categories.

① Spherical Shaped (Coccus)
② Cylindrical or Rod Shaped (Bacillus)
③ Spiral Shaped

SPHERICAL

- These are basically Coccus bacteria which is spherical in shape
- They can be further classified as follows :

① Micrococcus : Singular Form , Mostly Gram tve & Non Flagellated.
② Diplococci : Present in the pair
③ Streptococci : Present in chains.
④ Staphylicocci : Present as a bunch of grapes.
⑤ Tetrads : Present in group of 4.
⑥ Sarcinae : Present in 3 planes as a cube.



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ROD SHAPED

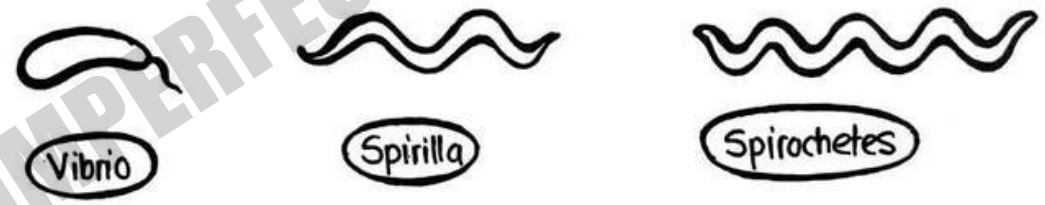
- These are basically bacillus bacteria that is cylindrical in shape.
- They can be further classified as follows :

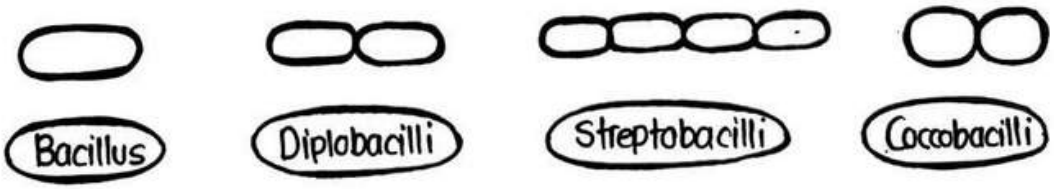
① Bacillus : Singular form
② Diplobacillus : Present in a pair
③ Streptobacilli : Present in a chain
④ Coccobacilli : Intermediate b/w Cocci & bacilli

SPIRAL

- These bacterias are helical-shaped having 1 or more twists.
- They are sub-classified as follows :

① Vibrio : Comma shaped.
② Spirilla : Coil Shaped.
③ Spirochetes : spiral coil shaped.





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NUTRITIONAL REQUIRMENTS OF BACTERIA

- Bacterias differ widely in their nutritional requirments :
- Given below are some common nutrients that are most necessarily required for the growth of bacteria.

① Water
② Carbon
③ Nitrogen & Sulphur
④ Oxygen
⑤ Organic Growth Factors

WATER

- It is the major nutrient forming 80-90% total weight of bacteria.
- Water (H_2O) provides Hydrogen & Oxygen that are essentially used for many activities of bacteria.
- Tap water contains Manganese, Cobalt, Copper, Nickel, Calcium etc that is also required as Micronutrients for the growth of bacteria.

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CARBON

- Bacteria uses CO_2 as primary source of carbon.
- It is the major constituent of cellular material.
- Autotrophs completely depends on CO_2 as source of carbon while Heterotrophs required organically combined carbon such as Glucose, Amino Acids as carbon source.

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NITROGEN & SULPHUR

- Bacteria requires large amount of Nitrogen & Sulphur for synthesis of all the key constituents of bacteria & for bacterial growth.
- These elements are used in reduced form such as Nitrogen as in amino acids & Sulphur as in Sulphydryl Compounds.

OXYGEN

- Oxygen is the major key nutrient for Aerobic Bacteria.
- Aerobic bacteria requires oxygen to grow because their methods of energy production & respiration depends on the transfer of electrons to oxygen. in the Electron Transport Reaction.

ORGANIC FACTORS

- These are essential organic compounds that are used as precursor by bacteria for synthesis of biomolecules like Protein, Nucleic Acid etc & enzymes.

① Amino Acids : Required as constituent of Protein
② Purines & Pyrimidines : Required as constituent of Nucleic Acids.
③ Vitamins : Forming part of prosthetic groups or active centres of certain enzymes.

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CULTURE MEDIA

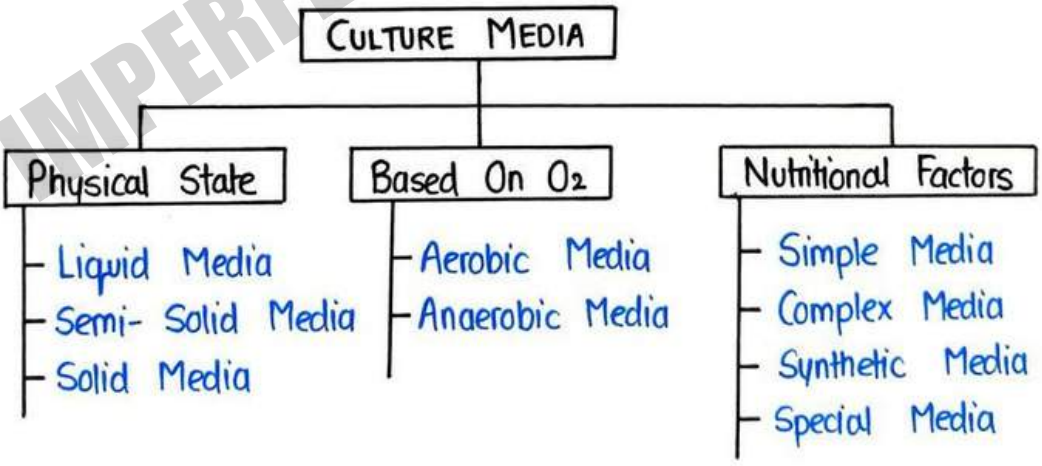
Culture Media is a type of medium of mixture of various nutrients and minerals required for growth and multiplication of various Microorganism.

NEED FOR CULTURE MEDIA

- To identify the characteristics of Microorganism.
- To differentiate among different kinds of bacteria.
- To test the effect of antibiotic drugs.
- To identify the cause of infection.
- For certain Morphological & Genetic studies.

CLASSIFICATION OF CULTURE MEDIA

Culture Media can be classified as follows :



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RAW MATERIALS USED IN CULTURE MEDIA

The various raw materials used for the preparation of Culture Media are as follows :

① Water
② Electrolytes
③ Peptones
④ Meat Extracts
⑤ Blood or Serum
⑥ Agar

WATER

- It acts as a solvent for the preparation of most of the culture media.
- Most of the enzyme controlled reactions occurs within cell in the presence of water.
- It also serves as source of Hydrogen & Oxygen.
- Tap water or Pure water is generally used, Distilled water can't be used in culture media.

ELECTROLYTES

- Electrolytes provides basic elements to the cell that are essential for fundamental metabolic process of Microorganisms.
- Generally Sodium Chloride used as source of Electrolyte.

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PEPTONE

- They are water soluble products obtained from Protein Hydrolysis.
- They are Hygroscopic in nature.
- Peptones are basically partially digested proteins prepared from animal or vegetable extracts.
- It acts as a buffer.
- It serves as source of Protein, Amino Acids & Various Inorganic Salts.

MEAT EXTRACT

- It is prepared from Fresh lean meat.
- It contains Gelatin, Creatin, Proteins, Amino Acids, Carbohydrates, inorganic salts and various Growth Factors.

BLOOD OR SERUM

- Blood and Serums are also used in the preparation of various Culture Medias.
- Usually 5-10% defibrinated Sheep blood is used.

AGAR

- Agar is basically used as Solidifying Agent.
- It is a long chain polysaccharide prepared from Seaweed (Algae).
- It contains small amount of proteins & variety of Inorganic Salts.
- It is used in concentration of 2-3 %.
- It melts at 98°C & solidifies at 42°C.

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Types Of Culture Media

Culture Media can be classified on the basis of 3 categories :

① On the basis of Physical State
② On the basis of presence or absence of O_2
③ On the basis of Nutritional Factors.

① On The Basis Of Physical State

On the basis of Physical state Culture Media can be classify as follows:

- Solid Media
- Semi - Solid Media
- Liquid Media

Solid Media

- Solid culture media is prepared by adding 1.5 - 2% Agar.
- Solid Culture Media are used to grow microorganism in their full physical form or prepare bacteria in pure cultures.

Liquid Media

- Liquid Media don't have addition of Agar.
- This media shows the growth of large number of bacteria.

Semi - Solid Media

- This Media contains Agar at 0.5% concentration or less.
- The growth of bacteria in semi - solid media appears as a thick line in the medium.

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② On The Basis Of Presence & Absence Of O_2

On the basis of O_2, it is classified into two types

- Aerobic Culture Media
- Anaerobic Culture Media

Aerobic Media

- Aerobic Culture Media are used to grow Aerobic Microorganisms.
- It is a rich & complete media used to grow microbes in bulk.
- Example : MacConkey's Broth.

Anaerobic Media

- Anaerobic Culture Media are used to grow Anaerobic Microbes.
- Anaerobic Media contains extra nutrients like Vitamin k, Hemin, Glucose, Thioglycolate, Cysteine etc.
- Example : Robertson's Cooked Meat Broth.

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③ On The Basis Of Nutritional Factors

On the basis of Nutritional requirments, baeter culture media can be classified as follows :

- Simple Media
- Complex Media
- Synthetic Media
- Special Media

Simple Media

- It is also known as Basal Media.
- This media is mainly used for the growth of Non-Fastidious Microbes.
- It is the most common media used for study of pathogenic bacteria.
- Example : Nutrient Agar / Peptone Water.

Complex Media

- Media other than simple media are known as Complex Media.
- They have added ingredients such as Yeast extract, casein etc
- It consist a mixture of many chemical species in unknown proportion.

Synthetic Media

- These Media are prepared using pure chemicals and used for
- special studies such as Metabolic Requirments.
- Example : Peptone Water.

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Special Media

Special Culture Media can be further sub-classified as follows :

① Enriched Media
② Enrichment Media
③ Selective Media
④ Differential Media
⑤ Indicator Media
⑥ Transport Media
⑦ Sugar Media

ENRICHED MEDIA

- When basal media is added with some nutrients such as Blood, Serum or Egg then it is called Enriched Media.
- They are used to grow bacteria that having more nutritional needs.
- Example : • Blood Agar (contains blood)
  • Chocolate Agar (Heated blood agar)

ENRICHMENT AGI MEDIA

- This media is used to increase the growth of specific microorganism.
- It is a liquid media that either stimulates the growth of bacteria of our interest or inhibits its competitors.
- Example : Selenite F Broth, Alkaline Peptone Water.

SELECTIVE MEDIA

- Selective Media is same as Enrichment Media, the only difference is, it is a Solid Media
- Example : Thayer Martin Agar, TCBS Agar.

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DIFFERENTIAL MEDIA

- This media contains some substances that helps us to differentiate between different characteristics of bacteria.
- Example : MacConkey's Agar.
- MacConkey's Agar shows Lactose Fermenter as Pink Colonies while Non Lactose Fermenter as Pale / Colourless Colonies.

INDICATOR MEDIA

- This media contains indicators which changes colours when bacteria grow on them.
- Example : Wilson & Blair Medium.

TRANSPORT MEDIA

- This media is used for transporting the samples.
- They are basically used for temporary storage of Microbes that are being transported to the laboratory for cultivation.
- It prevents the sample and inhibits the overgrowth of unnecessary bacteria.
- Example : Pike's Media.

SUGAR MEDIA

- It contains 1% sugar which can be any fermentable substance such as Glucose, Mannitol, Sucrose, Lactose etc.
- Example : Mannitol Agar.

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PHYSICAL PARAMETERS FOR BACTERIAL GROWTH

- Physical Parameters For Bacterial Growth can also be known as Factors Affecting Bacterial Growth.
- Factors affecting bacterial Growth are as follows :

① Nutrition Concentration
② Temperature
③ Gaseous Concentration
④ pH
⑤ Ions & Salt Concentration
⑥ Water

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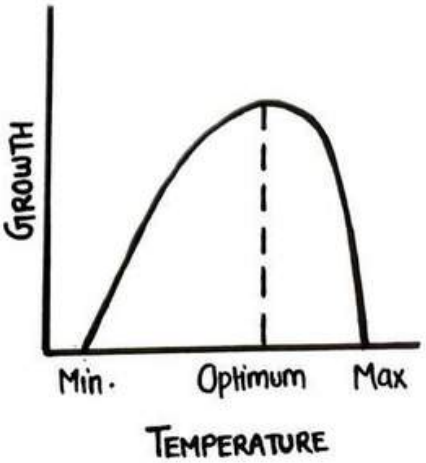
NUTRITION CONCENTRATION

- Bacterial growth is directly depends on the available nutrients.
- If Culture Media is rich in nutrients, growth of bacteria occurs faster & vice- versa.
- Nutritional requirments varies with bacteria species.

TEMPERATURE

- Temperature also plays an important role in bacterial growth.
- The temperature at which bacterial growth is maximum, known as Optimum Temperature.
- The lowest temperature that allows bacterial growth is called Minimum Temperature.
- The highest temperature that allows bacterial growth is called Maximum Temperature.
- There is no growth below minimum & above maximum temperature.



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pH

- pH affects the ionic properties of bacterial cell.
- Most of the bacteria, grows at neutral pH (6.5-7.5), however there are certain bacterias that grows best at acidic or basic pH.
- Microorganism can be placed in one of the following groups based on their pH requirments.

Neutrophiles : Grows at pH 5 to 8

Acidophiles : Grows at pH below 5.5

Alkaliphiles : Grows at pH above 8.5

Ions & Salt

- All bacteria requires metal ions such as K^{+}, Ca^{2+}, Mg^{2+}, Fe^{2+} etc to synthesize enzymes & protein.
- Most bacteria requires Isotonic or Hypotonic environment.

Gaseous Requirments

- Oxygen & Carbon-di oxide are most important gases that affects the growth of bacteria.
- Oxygen is very important for growth of Aerobic Bacterias.

Water

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

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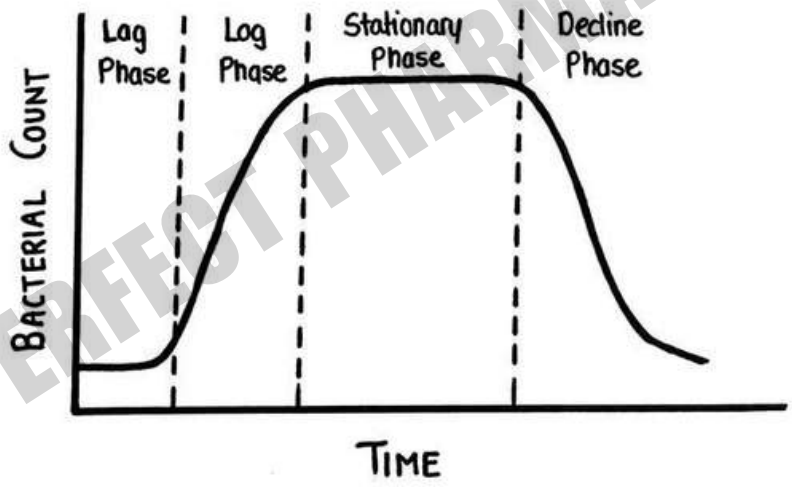
GROWTH CURVE

- When we pour bacteria into culture media, then it shows a definite growth pattern in that Medium.
- Bacterial Growth Curve is nothing but the Graph plotted between Bacterial Count in that Culture Medium with respect to Time.
- The bacterial growth curve consist of 4 phases :

① Lag Phase
② Log Phase
③ Stationary Phase
④ Decline Phase



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LAG PHASE

- Generally, multiplication of bacteria does not begin immediately after the introduction in culture media.
- In the lag phase, the bacteria adopts itself to the new environment.
- During this phase, the enzymes and intermediate metabolites are build up which are required for multiplication.

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LOG PHASE

- It is a growth period of bacterial cells in culture medium.
- During this phase, there is an exponential increase in the number of bacteria.
- Reproduction & Growth of bacteria are at their highest level in this phase.

STATIONARY PHASE

- During this phase, the rate of growth of cells becomes equal to rate of its death.
- In this phase, nutrients get exhausted & toxic products get accumulated.
- The total number of living cells becomes constant in this phase.

DECLINE PHASE

- It is also known as Death Phase.
- In decline phase, the bacterial population starts decreasing due to death of cells.
- The decline phase starts due to exhaustion of nutrients and accumulation of toxic products.

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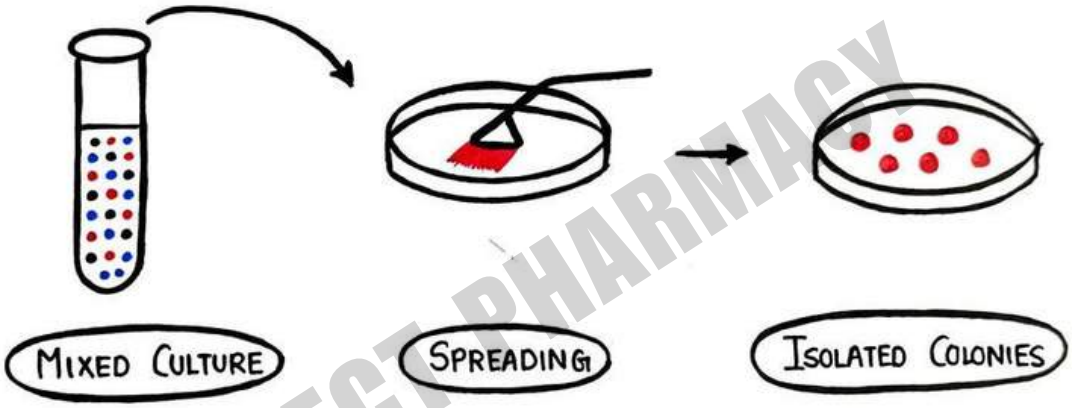
SPREAD PLATE METHOD

- This is the best method to isolate pure culture.
- In this technique 0.1 ml of sample taken from diluted mixture which is then placed on the surface of agar plate & spread evenly over the surface by using L shaped Glass Rod called Spreader.
- Thereafter, the medium is incubated.
- After incubation, colonies are observed on the Agar Surface.



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POUR PLATE METHOD

- In this medium first we take our sample in petri plates after that we add the liquid agar medium.
- After mixing the sample and liquid agar medium on petri-plates, we allowed them for solidifying & then incubated.
- After incubation, bacterial colonies appear on surface.

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Isolation Of Pure Culture

- Culturing is a procedure during which microorganism are cultivated in a culture media.
- Now, Generally when we culture microbes, we obtained a Mixed Culture, that contains more than 1 microorganism.
- Pure Culture is a type of culture medium that contains only 1 type of microorganism.
- Pure Culture is usually derived from mixed culture by transfering a small sample into a newly prepared culture media.
- There are following techniques by which we obtained a Pure Culture :

① Streak Plate Method
② Spread Plate Method
③ Pour Plate Method
④ Serial Dilution Method

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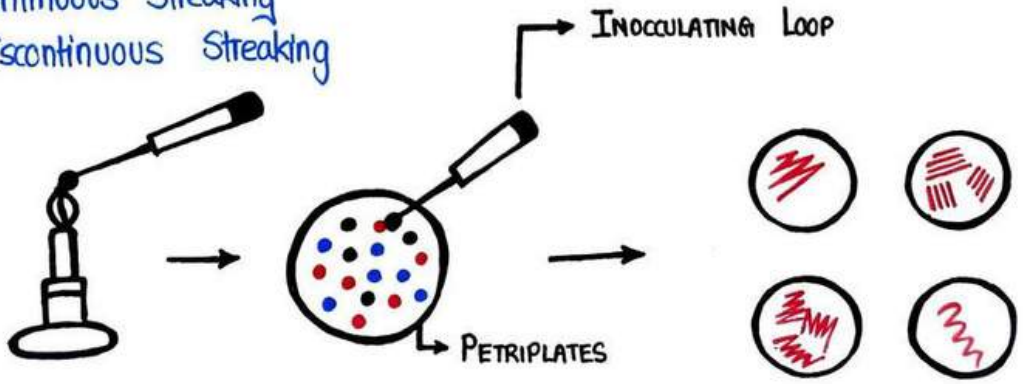
Streak Plate Method

- It is the most widely used method for isolation of pure cultures.
- Streaking is the process of spreading the microbial culture using an inoculating needle. on the surface of new media.
- In this, first we sterlize the inoculating loop by flame after that we streak out the bacteria using it on petri plates.
- It can be done by two types :

① Continuous Streaking
② Discontinuous Streaking



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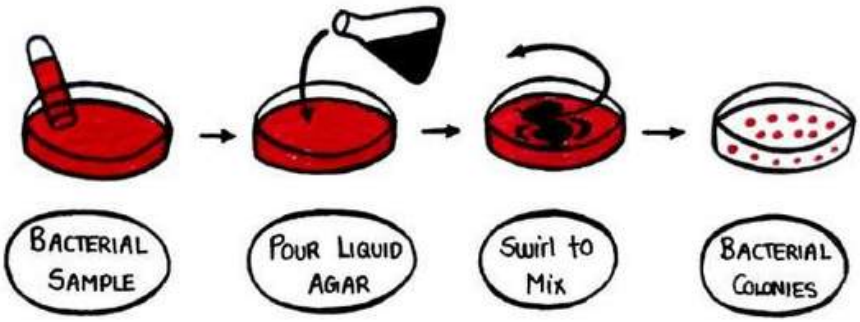
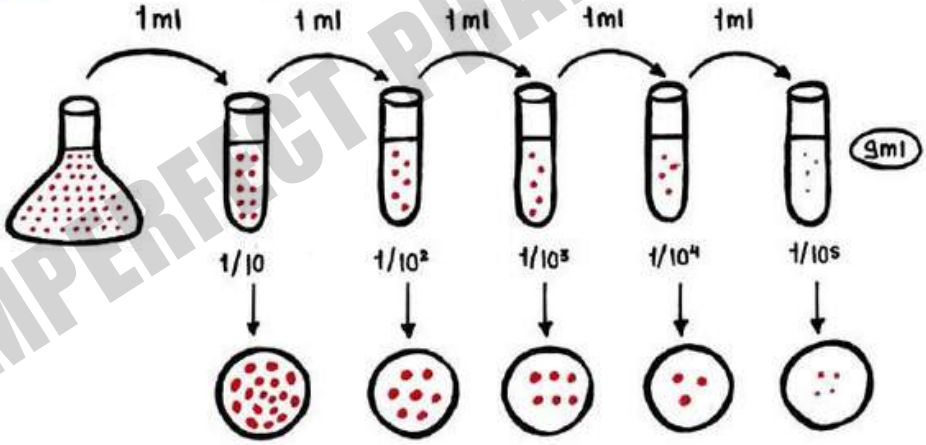


Serial Dilution Method

- Serial dilution method is one of the most commonly used technique for the isolation of pure culture.
- It is a stepwise dilution of a sample in a solution to reduce a highly concentrated solution in a dilute solution.
- The dilution blanks are prepared by having tubes filled with an appropriate dilution liquids.



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PRESERVATION OF PURE CULTURE

- After isolating the pure culture, it becomes very important to preserve the purity & viability of Pure Culture.
- Preservation of Pure Culture refers to maintain the stability & purity of pure cultures for longer period of time.

NEED OF PRESERVATION

- To maintain the stability & purity of Pure Cultures.
- To avoid contamination (impurity).
- To restric Genetic Mutation.
- To study about structural & functional properties.
- To preserve the bacteria having economical importantce for human beings & plants.

METHODS OF PRESERVATION

① Subculturing
② Refrigeration
③ Paraffin Coating
④ Cryopreservation
⑤ Lyophilization

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SUBCULTURING

- Pure Cultures can be easily by continuous transferring to a Fresh Media at a definite Time - Interval.
- It is an easy & simple method as it doesn't require any special apparatus or nutrients.
- Although this method is very simple & easy yet it is somehow time consuming & having risk of contamination.

REFRIGERATION

- The Pure Cultures can be maintained & preserved under the refrigeration temperature of 0-4°C.
- Refrigerators preserve the bacterial culture for upto 2-3 weeks & fungal cultures for 3-4 months.
- The Refrigeration temperature greatly slows down the metabolic activities of microorganism
- It is mainly used for Short - Time Preservation.

PARAFFIN COATING

- It is a simple & economical method of maintaining pure cultures.
- In this method sterile liquid paraffin is poured over the culture medium & stored at room temperature.
- The layer of paraffin ensures anaerobic conditions & prevents dehydration of medium.
- Paraffin Coating Method can preserve the bacterial culture for Months to Years.

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CRYOPRESERVATION

- This method is mainly used for preservation of Pure Cultures for a long period of time.
- In this method, the microorganism of culture are rapidly frozen in liquid Nitrogen at -196°C in the presence of stabilizing agents such as Glycerol or Dimethyl Sulfoxide that prevents the cell damage due to formation of ice crystals & promotes cell survival.
- By this method, Cultures can be preserved for 10-30 years.

LYOPHILISATION

- It is also known as Freeze-Drying method.
- Lyophilisation is done by using Sublimation Process.
- In this process the culture is first frozen at -60,-70°C & then major portion of water & solvent is reduced.
- It is a simple & cost effective method used for preservation of bacterial culture for long time.

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CULTIVATION OF ANAEROBES

- As we know that Anaerobic bacteria are those that can only be survived in the ~~presence~~ absence of Oxygen.
- Cultivation of Anaerobes stands for making a culture environment for Anaerobic Bacterias.
- For this we basically remove any existing oxygen in the system & reduce the oxygen content in Culture Medium.
- Following methods are most commonly used for cultivation of anaerobes:

① Candle Jar Method
② Anaerobic Jar Method
③ Anaerobic Chamber Method

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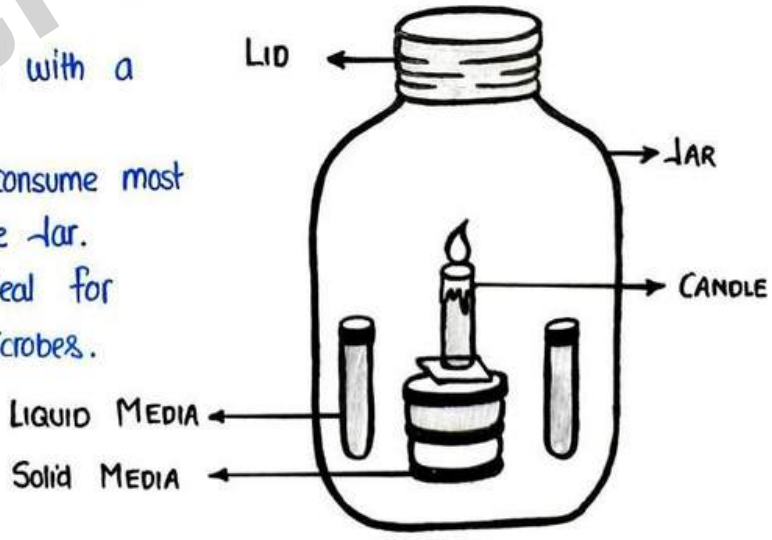
CANDLE JAR METHOD

- In this method Media plates kept inside the Jar.
- Afterthat, Jar is sealed with a burning candle inside.
- The candle flame will consume most of the oxygen inside the Jar.
- These conditions are ideal for growth of Anaerobic Microbes.



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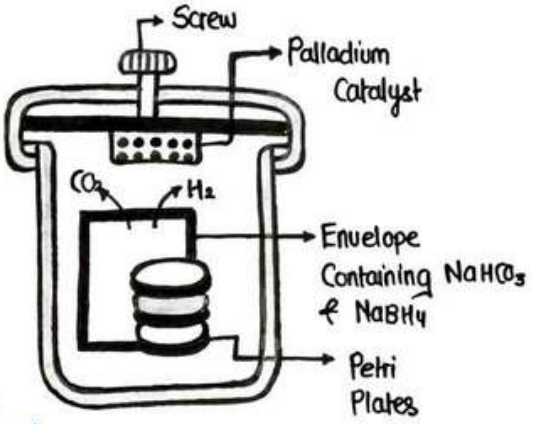
ANAEROBIC JAR METHOD

- In this method Media plates kept inside the jar containing Palladium Plates.
- It carries an envelope containing $NaHCO_3$ & $NaBH_4$, by their reactions CO_2 & H_2 are generated.
- The Hydrogen reads with oxygen present inside jar & converts into H_2O.
- The Anaerobic conditions can be confirmed by Methylene Blue indicator, it becomes colourless in absence of oxygen.



ANAEROBIC CHAMBER

- It is an Anaerobic Incubation System.
- It provides oxygen free atmosphere.
- It is fitted with Air tight Gloves to insert hand for working.
- The chamber contains sealed environment of H_2, CO_2 or N_2

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QUANTITATIVE MEASURMENT OF BACTERIA

- When a bacteria is inoculated into a culture media, then its population is generally counted at different time-intervals.
- There are various methods of Quantitative Measurment of Bacterial Growth as follows :-

① Total Count / Direct Method
- Counting Chamber Method
- Electronic Cell Counter

② Viable Count / Indirect Method
- Plate Count Method
- Turbidity Method

TOTAL COUNT

- In this method, we basically count the total number of bacterial cells (including living & death cells)
- It includes :

(i) Counting Chamber Method
(ii) Electronic Cell Counter

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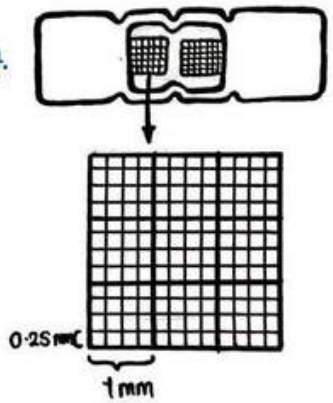
COUNTING CHAMBER METHOD

- It is also known as Haemocytometer Method.
- In this method we basically used a Haemocytometer placed on a glass slide
- First liquid media is spread over the chamber then the number of bacterial cells can be calculated by counting av. number of bacteria in these squares.



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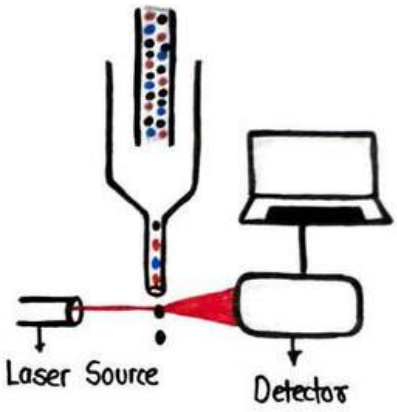
Electronic Cell Counter Method



- This method typically involves the use of an electronic cell counter or Flow Cytometer.
- The liquid media is passed through a capillary tube that ends with a microscopic diameter through which only one cell can pass at a time.
- A Laser beam is passed through bacterial cells that shows scattering on detector and readings are calculated by a Digital Reader.

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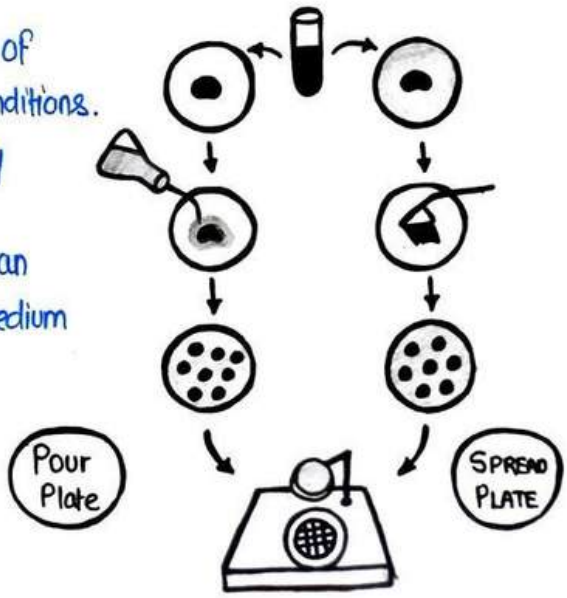
Viable Count

- In this method, we only count the living bacterial cells.
- It basically includes :

(i) Plate Count Method
(ii) Turbidity Method

Plate Count Method



- This method determines the number of cells that will multiply under certain conditions.
- It can be done by two ways either by Spread Plate or Pour Plate Method.
- In this method only those bacteria can be counted which can grow on the medium used & conditions provided.
- It is a time taking process.

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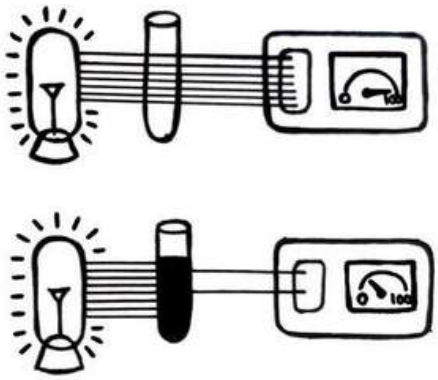
Turbidity Method

- It is commonly used indirect method for bacterial cell count in a liquid media.
- This method is based upon principle that more the number of bacteria, greater its turbidity in sample.
- The spectrophotometer basically counts the amount of light transmitted through the sample.
- Bacterial Count $\propto$ 1/ Transmittence.



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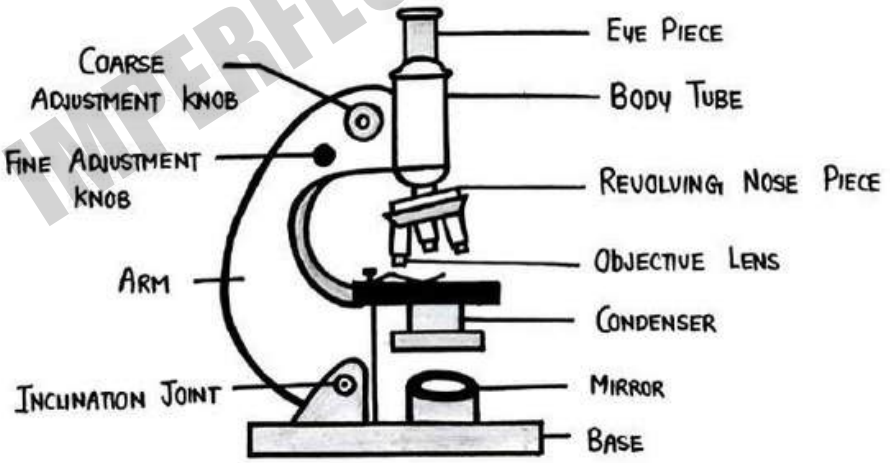
MICROSCOPY

- Microscopy is a scientific technique that involves the use of Microscope to view the objects that are too small to be seen with naked eyes.
- A Microscope is an instrument which consist of Lens or combination of lenses used to magnify the objects that cannot be seen with naked eyes.
- It utilises light (either sunlight or artificial light) or electron beam to see the enlarged images of object.

LENSES IN MICROSCOPES

A Microscope mainly consist of two types of Lenses :
Ocular Lenses (Eye Piece)
Objective Lenses

COMPONENTS OF MICROSCOPE



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MAGNIFICATION

- First Magnification → Objective Lens
- Second Magnification → Ocular Lens
- Final Magnification = FM X SM

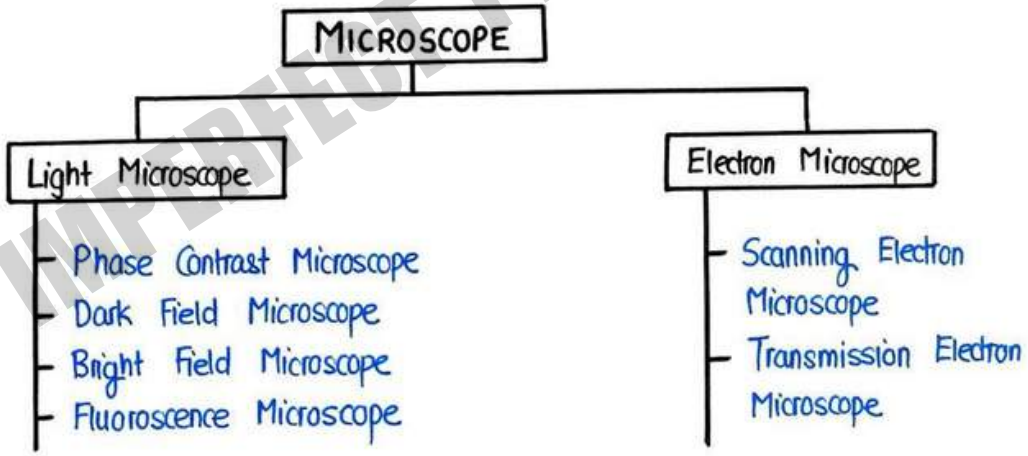
RESOLUTION

- In microscopy, resolution refers to the ability of a microscope to distinguish two points as separate entities.
- Higher resolution means finer details of specimen can be observed.

TYPES OF MICROSCOPY

Microscopy is of mainly two types:

① Light Microscopy
② Electron Microscopy



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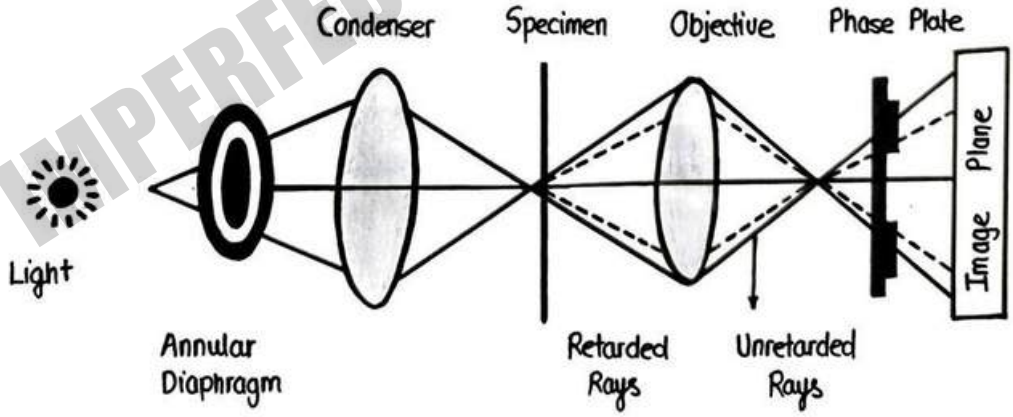
PHASE CONTRAST MICROSCOPE

- This Microscope was invented by a Dutch Physicist Frits Zernike in 1934. He was also awarded by Nobel Prize in 1953 for this.
- It is a microscope that allows observation of living cell.
- It is a type of microscope that allows the observation of transparents objects like living cells by converting phase shifts in light & enhancing contrast.
- With the help of phase contrast microscope, we can identify transparent objects without staining unlike Bright Field Microscopy.

PRINCIPLE

The Principle of Phase Contrast Microscopy involves conversion of small phase changes in light rays, induced by differences in thickness & refractive index of different parts of an object, into visible changes in brightness.



PHASE CONTRAST MICROSCOPE

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KEY COMPONENTS & WORKING MECHANISM

- **Light Source** : The microscope uses a bright light source, typically a Halogen or LED Lamp to illuminate the specimen.
- **Annular Diaphragm** : It is located in the condenser and adjust the light to come from all directions.
- **Phase Plate** : It is located in objective lens.
  The phase plate introduces a phase shift to the light that passes through it that causes a constructive and destructive interference.
- **Image Formation** : The interference between light waves results in differences in brightness & contrast in Final image. This makes the details of sample more visible.

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APPLICATION

- It helps to see details in living cells without staining them.
- It is used for visualisation of various cell organelles like Mitochondria, Nucleus etc.
- It is used to examine cells in medical samples for abnormalities.
- It allows observation of Bacteria & other tiny microorganisms.

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ADVANTAGES

- No need for staining.
- Provides high contrast images of cells.
- Cost Effective
- Useful for study of living cells & dynamic processes.

DISADVANTAGES

- It generally offers low resolution compared to some other microscopes like electron microscope.
- It may not work well with very thick & dense specimen.

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DARK FIELD MICROSCOPE

- A Dark Field Microscope is a type of Microscope that makes it easy to see tiny or transparent objects.
- It adjust light on sample in such a way that object itself appears bright while the background will be dark, almost black.
- This technique highlight details that might be hard to see with regular microscopes.

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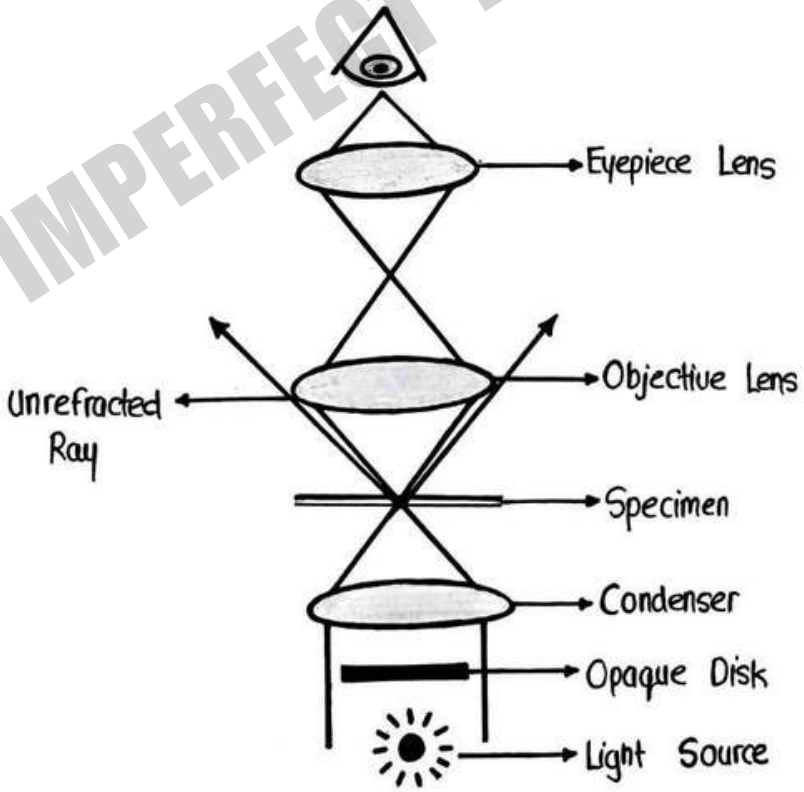
PRINCIPLE

The principle of Dark Field Microscope involves creating a bright image of specimen against a dark background by using angled light through an opaque disk.



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KEY COMPONENTS & WORKING MECHANISM

- **Light Source** : It provides illumination typically a bright focused beam of light.
- **Dark Field Condenser** : It is a special condenser consist of an Opaque Disk that blocks direct light from entering the objective lens
- **Scattering** : When the sample is illuminated, it scatters the light. This scattered light now coming from different angles is collected by objective lens.
- **Image Formation** : The objective lens focuses this scattered light to form a bright image of specimen against a dark background.

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APPLICATION

- It helps scientist see live cells & bacteria without needing to add dyes.
- It is used to view very small particles like nanoparticles to check their size & shape.
- It allows doctors to see fine details in tissue samples that are hard to spot with regular techniques.

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ADVANTAGES

- It enhances contrast of image
- Not required staining.
- It is effective for visualising very fine details.

DISADVANTAGES

- The overall image may appear dimmer compared to bright field microscopy because it relies on scattered light rather than direct light.

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ELECTRON MICROSCOPE

- An Electron Microscope is a microscope that uses a beam of accelerated electrons instead of visible light as a source of illumination.
- The wavelength of electrons can be upto 100,000 times shorter than that of visible light.
- It is of two types

① Transmission Electron Microscope

② Scanning Electron Microscope

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TRANSMISSION ELECTRON MICROSCOPE

Transmission Electron Microscope is a high resolution imaging technique that uses beam of electrons transmitted through an ultra thin specimen to produce detailed images.

PRINCIPLE

Transmission Electron Microscopy operates on the principle of transmitting a beam of electrons through a very thin specimen and forming an image based on the interactions between the electrons and the sample.

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WORKING MECHANISM

The working mechanism of Transmission Electron Microscopy involves several key steps :

- Sample Preparation
- Electron Source (Electron Gun)
- Beam Transmission
- Image Formation
- Image Detection
- Image Analysis

APPLICATION

- Transmission Electron Microscopy is used to observe fine structure of materials at nanoscale, including the study of crystal structures, defects and interfaces.
- It assist in diagnosing disease by examining cellular structures and abnormalities.

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SCANNING ELECTRON MICROSCOPY

Scanning Electron Microscopy is a powerful imaging technique used to observe the surface structure of samples at high magnification.

WORKING MECHANISM

The working of scanning electron microscopy involves several key steps :

- Sample Preparation
- Electron Gun as Electron Source
- Beam Focusing
- Scanning
- Interaction With Sample
- Signal Detection
- Image Formation

PRINCIPLE

The principle of scanning electron microscopy involves scanning a focused beam of electrons across the surface of a sample and detecting the emitted signals to create an image.

APPLICATION

- For analysis of surface features & roughness of materials like metals, polymers & ceramide.
- Examination of pathological samples such as tissues & biopsies to identify disease related changes.

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