

microbiology is the study of all living organisms that are too small to be visible with the naked eye. This includes bacteria, archaea, viruses, fungi, Prions, Protozoa and algae, collectively known as 'microbes'.

Introduction of microbiology

The study of small living things. micro = small, Bio = living, and logy = to study. microbiology (or specifically, bacteriology) is still a very young science and not yet completely understood. Only about three hundred years have passed since the discovery of the first bacteria. Many estimates suggest that we have studied only about 1% of all the microbes in any given environment. In the scope of the world, it is obvious to see that the discipline of microbiology is still in its infancy.

Living cells and how they work. microorganisms, an important class of cells capable of independent existence.

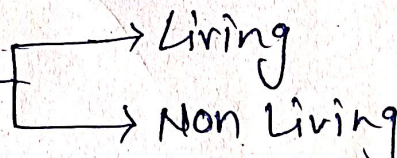
microbial diversity and evolution.

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What microbes do in the world, in human society, in our bodies, and in the bodies of animals and plants. It is about the central role microbiology plays as a basic biology science and how an understanding of microbiology helps in the understanding of the biology of higher organisms—including humans.

- * When microbiological concepts, process and techniques are applied to pharmaceutical operations, the subject is then 'pharmaceutical microbiology'.
- Objective of pharmaceutical microbiology to ensure efficacy safety of pharmaceutical product.

History of microbiology

Aristotle :- 

- They also told many living organisms are exist in huge number but we doesn't visualised.

Roger Bacon :- 13th century → Disease.

[∴ Any living organisms (very small) enters in our body to cause disease].

Antonie van Leeuwenhoek (1675) :-

- founder of microbiology.
- 1st person to see microbes and gave a name "Animalcules" $\left\{ \begin{array}{l} \text{Protozoa} \\ \text{Bacteria} \end{array} \right.$

Johan tyndall :- concept tyndall effect

- if we provide heat to any food substance bacteria doesn't survive or killed.

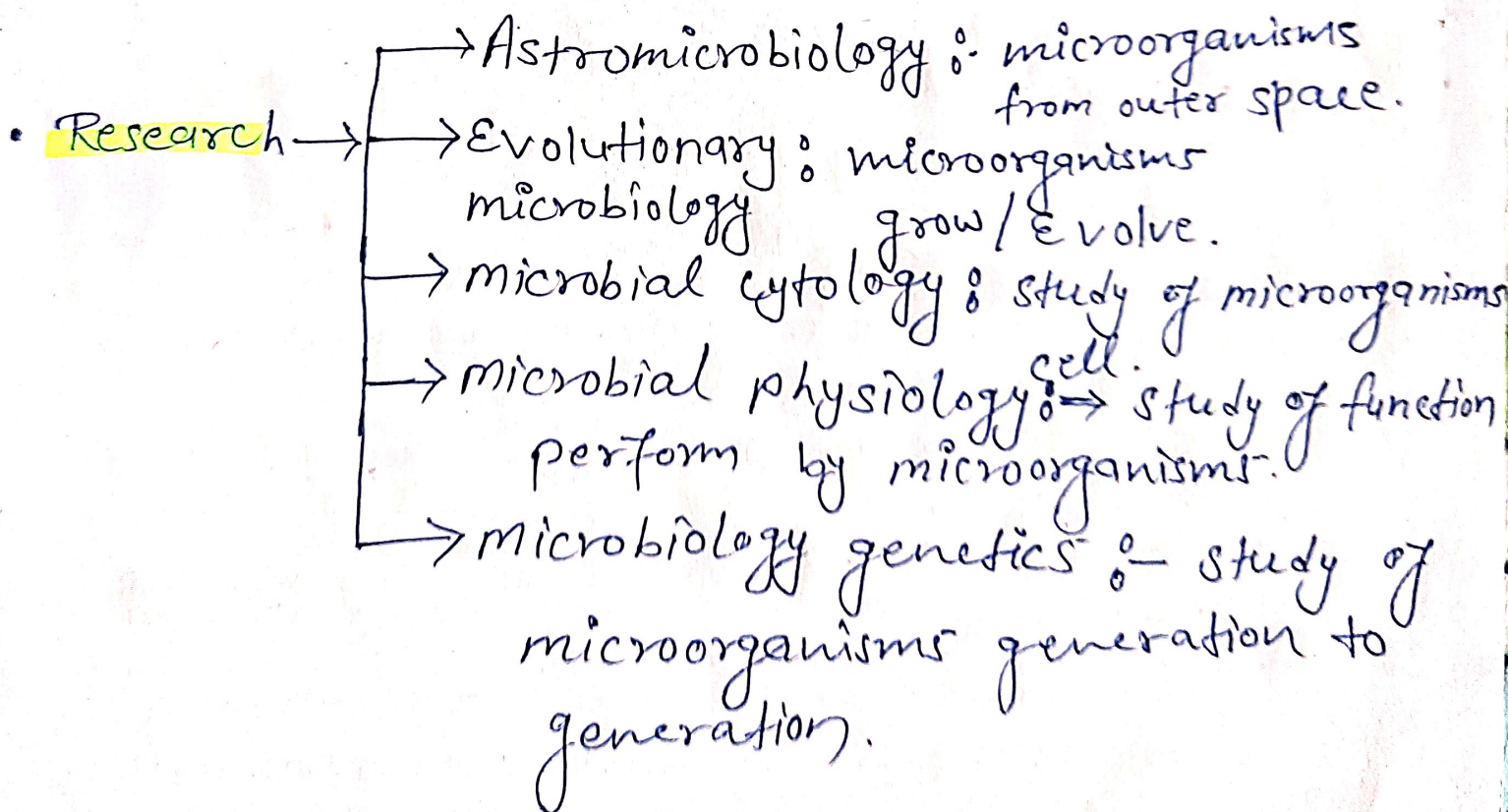
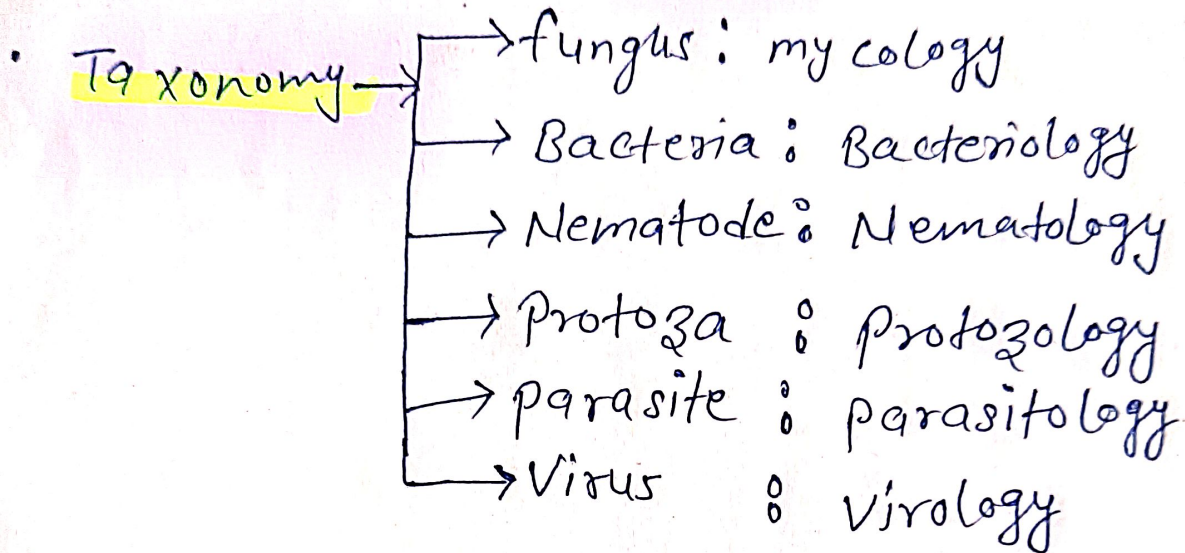
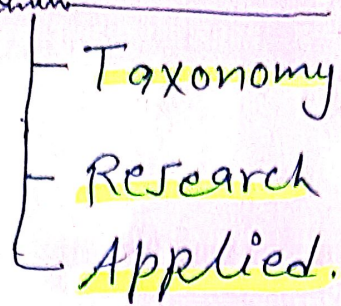
Louis pasture :- father of microbiology.

- Discover vaccines against chicken pox, rabies.
- They explain microbes.
- They also gave many terms $\left\{ \begin{array}{l} \text{microbiology} \\ \text{aerobic} \\ \text{anaerobic} \end{array} \right.$
- Pasteurization concept $\Rightarrow 62.8^\circ \rightarrow 30 \text{ min.}$

Alexander Flemming :- first antibiotic discover to kill bacteria.

- In 1928, they discovered penicillin.

Branches of microbiology



- Applied →
- Medical microbiology :-
 - microbes are used to produce antibiotic.
 - Pharmaceutical microbiology :-
 - microbes used in pharmaceutical products.
 - Industrial microbiology :-
 - microbes used in economic & industrial purpose.
 - Veterinary microbiology :-
 - study / applied on animals.
 - Agriculture microbiology :-
 - Genetic engineering is used for the production of transgenic plants.

Scope of microbiology

- The scope of microbiology is increasing day by day because of the advancement in the field of science and technology, the field in which microbiology are intensively used are as following :-

1. Food and dairy
2. Environmental microbiology
3. Immunology
4. Agriculture / farming
5. Industrial microbiology
6. Pharmaceutical microbiology.

1. Food microbiology :-

- The food microbiology is a branch of science that deal with the study and application of microorganism in food and dairy industry.
- microbiology finds its scope in the development of several products like cheese, pickle, yogurt etc. simply by the use of microorganism.

examples :-

1. *Candida colliculosa* is used in the preparation of cheese.
2. Yeast is used in preparation of ethanol.

2. Environmental microbiology :-

- It is the study of the function and diversity of microbes in their natural environments.
- Environmental microbiology finds its scope in following fields.

- (a) Analysis of waste bio treatment.
- (b) oil biodegradation

3. Industrial microbiology :-

- Industrial microbiology may be defined as the study of the large-scale and profit motivated production of microorganisms and their products for direct use, or as input in the manufacture of other goods.

Example :-

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Yeast may be produced for direct consumption as food for humans or as animal feed, or for use in bread-making.

- The product of yeast such as ethanol may be consumed in the form of alcoholic beverages, or used in the manufacture of perfumes.

4. Immunology :-

- It is the study of the immune system or more specifically it may be defined as a branch of microbiology that deals with the study of the development, anatomy, function and malfunction of the immune system.
- Immunology finds a great scope in developing potential vaccines against many disease by an intense study over different microorganisms.

5. Pharmaceutical microbiology :-

- It is the study of microorganisms that are related to the production of antibiotics, enzymes, vitamins, vaccines, and other P'ceutical products and that cause P'ceutical contamination and spoil.
- The P'ceutical microbiology finds a scope in the production of antibiotics as well as preventing the sterile and non sterile product from microbial contamination.

6. Agriculture / Farming :-

- There are many bacteria which are considered as to be good for the development and growth of plant for example, Azotobacter, Clostridium and Rhizobium are few bacteria whose job is to fix free nitrogen of the soil and make it available to the plants.
- microbiology find a great scope in providing high yield of raw food materials from the fields. by simply studying the properties and nature of the microorganism and using them in the agriculture fields.

Importance of microbiology

1. Production of antibiotic Eg. penicillin from penicillium.
2. Plant growth promotion
3. Sterile product preparation
4. Testing of pharmaceutical product and raw material.
5. Identification of microorganisms. Eg. morphological, cultural or microscopic study.
6. Treatment of industrial waste and material.
7. Production of enzymes, vaccines, alcoholic & other pharmaceutical products.

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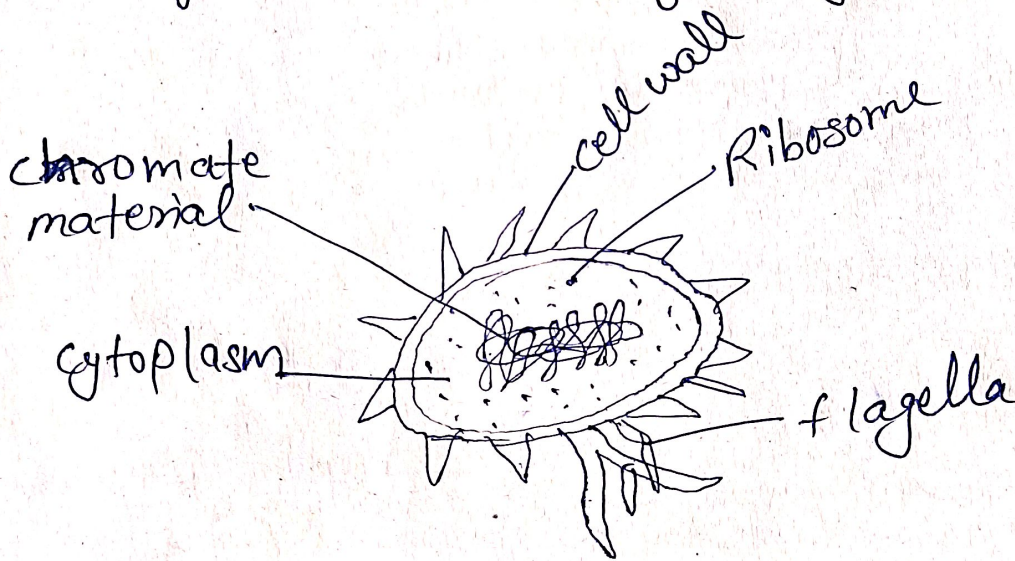
Prokaryotes and Eukaryotes

- All cells contains three feature in common
i) cell membrane ii) cytoplasm iii) DNA.

Prokaryotes

Prokaryotes are a microscopic single-celled organism.

- Pro - before & karyon - Nucleus
- origin 3500 million years ago.



Prokaryotic cell

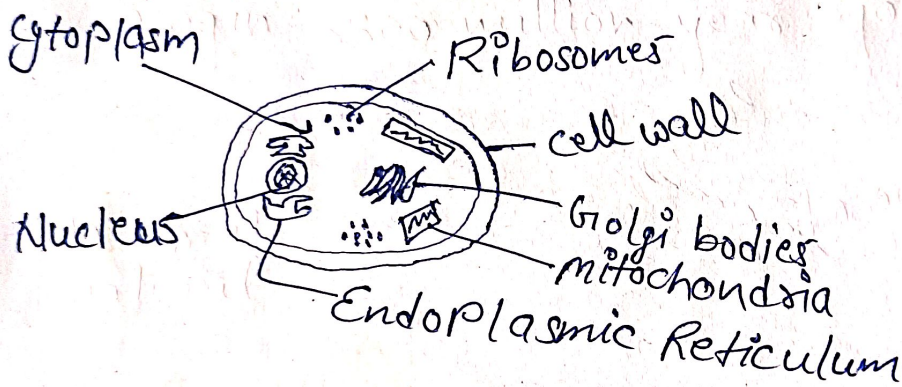
- Size $0.1 \mu m - 5.0 \mu m$
- Lack of Nuclear membrane hence Nucleus is absent.
- cell wall made up of murein or peptidoglycan
- chromosome composed of DNA.

Eukaryote

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A eukaryote is an organism with complex cells, or a single cells with a complex structures. In these cells the genetic material is organised into chromosomes in the cell nucleus. Animals, plants, algae, and fungi are all eukaryotes.

- Eu-True & karyon - nucleus
- origin 2500 million year ago.



Eukaryotic cell

- Size more than 10 μm
- Nuclear membrane & Nucleus is present.
- cell wall made up of cellulose (plant) and chitin (fungi).
- chromosome composed of protein & DNA.

Bacteria are single celled microbes. The cell structure is simpler than that of other organisms as there is no nucleus or membrane bound organelles. Instead their central centre containing the genetic information is contained in a single loop of DNA.

Some bacteria have an extra circle of genetic material called a plasmid.

- It was first observed by the scientist 'Antonie van Leeuwenhoek' in 1675.
- size is about 0.5 to 5 μm

Morphological classification of Bacteria

Bacteria are classified into five groups according to their basic shapes:

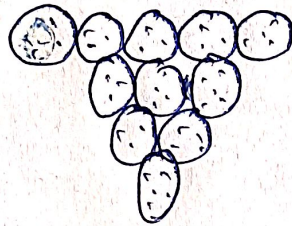
- Cocci
- Bacilli
- Spirilli
- Vibrios
- Spirochaetes

Cocci — These are spherical or round shape.

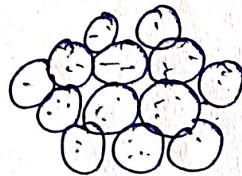
- It has many arrangement like —
 - (i) Monococci — Present in single cell.
eg. micrococcus. 
 - (ii) Diplococci — Present in a pair of two cells.
eg. Diplococcus Pneumoniae. 
 - (iii) Tetrads — Present in group of four.
eg. Staphylococcus tetragena 

(iv) Streptococci :- Present in chain round shape.
eg. streptococcus lactis.

(v) Staphylococci :- Present in irregular pattern like bunch of cells.
eg. staphylococcus aureus.

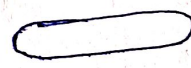


(vi) Sarcinae :- Present in cube like group.
eg. sarcina



Bacilli :- rod or cylindrical shape bacteria.

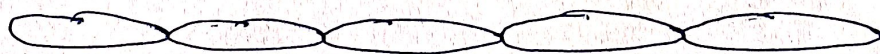
• It also has many arrangements :-

(i) monobacilli :- single rod shaped bacteria.
eg. Bacillus 

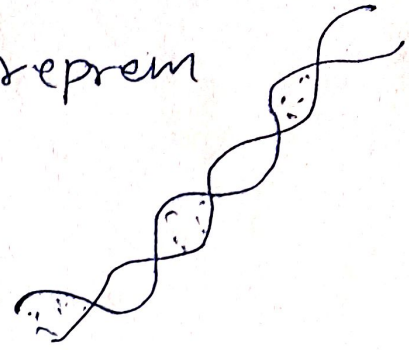
(ii) Diplobacilli :- Present in pair of two.
eg. Klebsiella pneumoniae.



(iii) Streptobacilli :- Present in chain rod shape.
eg. streptobacillus anthrax.



Spirilli :- These are spring or helical rod shaped bacteria.
eg. *Spirillum replem*



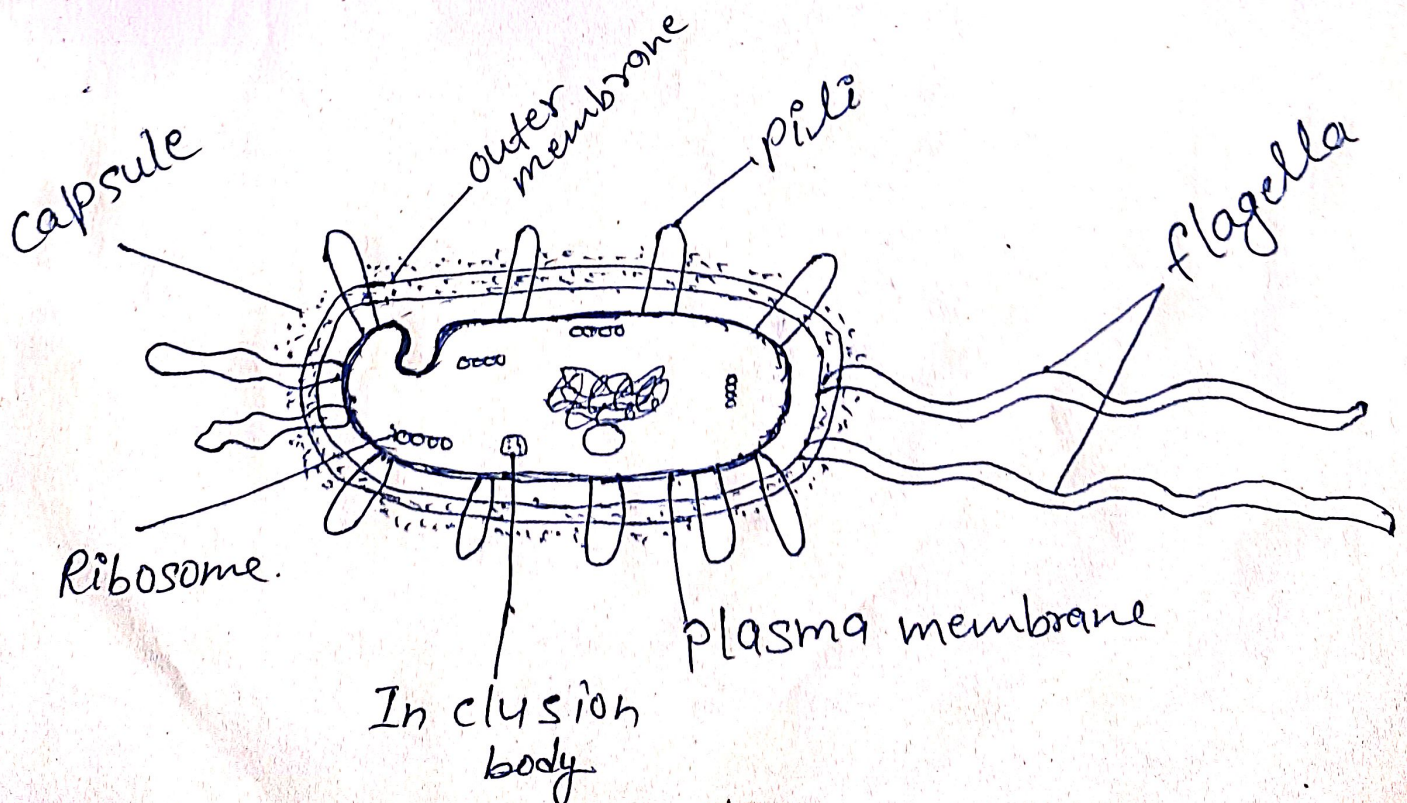
Vibrios :- Comma shaped bacteria.
eg. *Vibrio harveyi*



Spirocheter :- longer spiral shaped with several coiled shape bacteria.
eg. *Treponema pallidum*



Ultrastructure or structure of Bacteria :-



Capsule :-

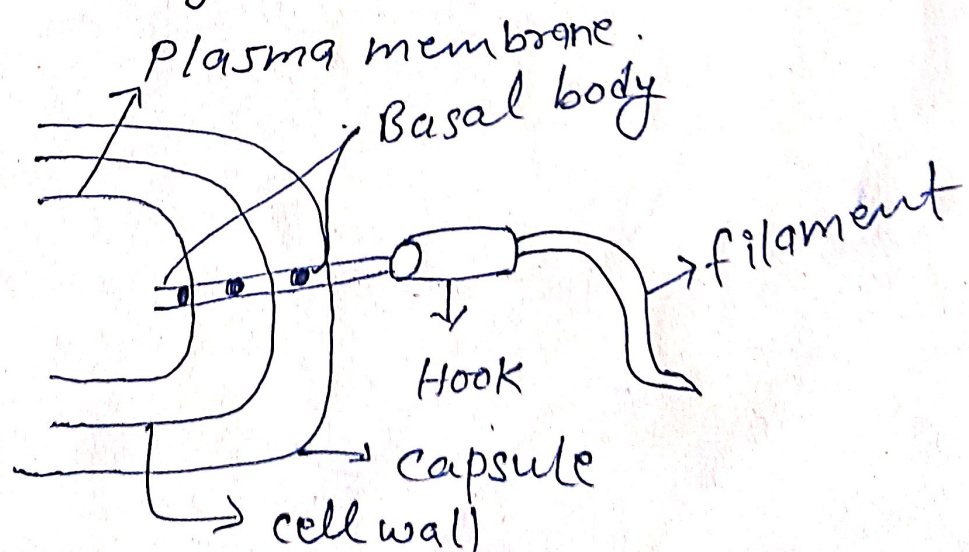
- The bacteria capsule is very large structure of any bacteria. It is a polysaccharide layer that lies outside the cell envelop.

function :-

- The capsule of bacteria enhance the ability of bacteria to cause disease.
- It provide protection from external environment.
- The capsule can protect cells from engulfment by Eukaryotic cell, such as macrophages.

flagella :-

- flagella are helical shaped structure which is composed of a protein called as flagellin.
- The flagella involves in locomotion means it help the bacteria to move from one place to another place by the use of few electrons.



Ultra structure of flagella

- It is composed of 3 parts :-
- 1. Basal body :- flagellum attach plasma membrane.
- 2. Hook :- Thick region of flagellum and pass out through cell wall.
- 3. Filament :- Thinner and terminal part of flagellum.

Types of flagella

- (i) Monotrichous  one end one flagella
- (ii) Lophotrichous  one end multiple flagella
- (iii) Amphitrichous  both end multiple flagella

Pili (fimbriae) :-

→ (extra organ)

- There are two types of hair appendage, they are present on the surface of bacteria.
- Pili is longer than fimbriae.
- The fimbriae contributes the bacteria ability to cause disease by binding on the surface of a cell also called as attachment pili.
- They are about 0.3 to 2 μm in length.

Cell wall :- The cell envelop is composed of plasma membrane & cell wall.

- The bacteria cell wall is different from the cell wall of other organisms because they contain Peptidoglycan layer that provide rigidity of bacteria cell wall & it the main structure component of cell wall.

Peptidoglycon also known as murein.

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Types of cell wall :-

i) Gram positive cell wall —

- Gram +ve cell wall are thick as compare to gram -ve cell wall.
- 95 % Peptidoglycan present.

ii) Gram Negative cell wall —

- Gram -ve cell wall thin as compare to gram +ve cell wall.
- Contain thin layer peptidoglycon layer adjacent to cytoplasmic membranes.

Internal structure

Cell membrane :-

- The bacterial cell is surrounded by a cell membrane which is primarily made up of double layer of phospholipid.
- It enclosed the content of cell and act as a barrier to hold them.
- It is also known as plasma membrane.

Cytoplasm :-

- It may be defined as viscous substance made up of cytosol (a gel like substance) which is present inside the cell.

- All the content of the cell (including DNA) of Prokaryotic organism are present within the cytoplasm.
- Most of metabolic pathway occurs in cytoplasm.

Ribosomes :-

- Bacteria Ribosomes are generally composed of two subunits 50s and 30s.
- Help in protein synthesis involve.
- During Protein synthesis both subunits combine & start the process.

11 # Nutritional Requirement of bacteria

- All the living organisms requires nutrition for their Proper growth and functioning.
- The major nutritional requirements of microbes are water, oxygen, Hydrogen, carbon, sulphur, phosphorous, PH, temperature, Osmotic pressure & Source of energy.

Source of Energy :-

- All the organism requires source of energy
- Photoautotrops :- Take energy from sunlight for food synthesis.
- Chemotrops :- depend on the chemical compound to get energy.

eg. In Rusting of Iron, ferrous is convert into ferric ion and release energy, so that energy is used by the bacteria to synthesised food.

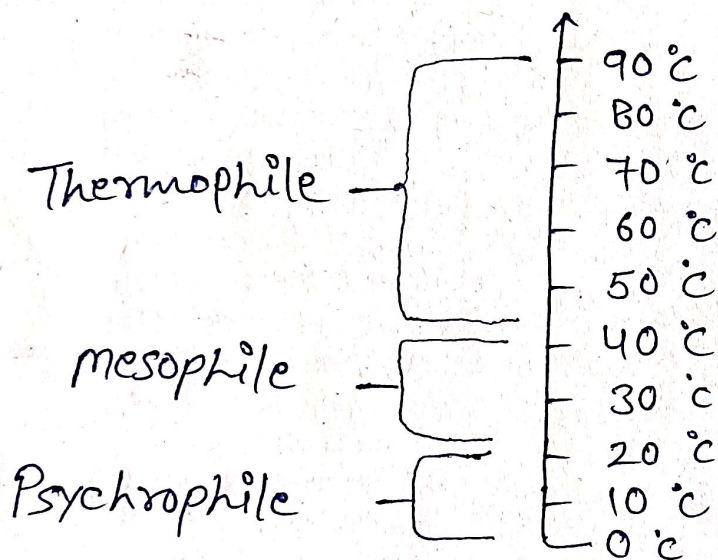
- * Water :- All living organisms requires water. In bacteria, with the help of water nutrients are convert into aqueous solution before they can enter the cells.
- * Oxygen :- main consituent of cell material. requiried all living organisms to build their biochemical component.
- * Sulphur :- sulphur is need for synthesised of certain amino acids i.e. methionine, cysteine, etc.

factors affecting bacteria

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* Temperature :-

- It is the most imp. factor that determines the rate of growth, multiplication and death of all living organisms.
- * High temperature damage microbes by denaturing enzymes.
- At very low temperature membrane also solidify and enzymes also do not function properly.



- * pH :- The pH is most essential for multiplication of micro-organism.
- The microbial growth is strongly affected by the pH of the medium.

Phosphorous $\rightarrow$ main component of nucleic acid, nucleotides, phospholipids, etc. 20

* **Nitrogen** $\rightarrow$ Living organisms require nitrogen for synthesis of cell components like amino acids, Purines, Pyrimidines, lipids, etc.

* **Hydrogen** $\rightarrow$ Important element or main source of organic compound.

* **Carbon** $\rightarrow$ skeleton / backbone of all organic molecules from which the organisms is built.

* **Temperature** $\rightarrow$ major environmental factor controlling microbial growth.

* **pH** $\rightarrow$ Each organisms has a pH range. many bacteria best grow in pH range 5.5-7.5.

* **Osmotic Pressure** $\rightarrow$ for survive and growth, microorganisms prefer isoosmotic.

Acidic	0	PH	
	1		} extreme acidophiles
	2		
	3		
	4		} acidophiles
	5		
	6		
Neutral	7		} Neutrophiles
	8		
	9		
	10		} Alkalophiles
	11		
	12		
	13		} extreme alkalophiles
Basic	14		

* water $\Rightarrow$ used as solvent in which Molecules are dissolved.

• Bacteria cells consists of 80% of water.

* Light $\Rightarrow$ phototrops bacteria usually take or requires light source for survive.

classification of culture media

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(A) On The basis of consistency

1. Solid medium :- solid medium contains agar at a concentration of 1.5 - 2.0 %. Solid medium is useful for isolating bacteria or for determining the colony characteristic of the isolate.
2. Semisolid media :- They are prepared with agar at concentrations of 0.5 % or less. They have soft custard like consistency and are useful for determination of bacterial motility.
3. Liquid medium :- These media contain specific amounts of nutrients but don't have gelling number of organisms, fermentation studies, and various other tests.
 - 0.5 % agar, Transportation or motility medium.

(B) On The Basis of composition

1. Natural medium :- It includes milk, urine, blood, meat extract, vegetable juice, peptone etc.
2. Synthetic or chemically defined medium :-
 - They are prepared from purified ingredients and their exact composition is known. E.g. Richard's solution.
3. Non synthetic or chemical undefined medium :-
 - Their chemical composition is partially known. They contain agar as a component. E.g. potato dextrose agar.

c) on the basis of purpose / functional use / Application (23)

1. General purpose media / Basic media :-

- Basal media are basically simple media peptone water, nutrient broth and nutrient agar are considered as basal medium. These media are generally used for the primary isolation of microorganisms.

2. Enrich medium (Added growth factors) :-

- Addition of extra nutrients in the form of blood, serum, egg yolk etc. to basal medium make them enriched media. eg. Blood agar, chocolate agar;

3. Selective and enrichment media :-

- * Both these media serve the same purpose. They selectively allow the growth of particular type of microorganism and prevent the growth of other type of microorganism.

(a) Selective media are agar based (solid). eg. MacConkey's agar.

(b) Enrichment media are liquid consistency. eg. Selenite F broth.

Raw material used for culture media ∴ page 24

⇒ A culture medium is a solid, liquid or semi-solid designed to support the growth of microorganism or cells.

⇒ A culture medium contains water, a source of carbon and energy, source of nitrogen, trace elements and some physical parameters or factors necessary for microbial growth. The pH of the medium must be set accordingly.

Raw ingredients used to make culture media ∴

1. protein source ∴ peptone (a complex mixture of partially digested protein). Eg. are Neopeptone and proteose peptone. Digest broths can also be used as meat extract.

2. mineral salt ∴ sodium chloride

3. Yeast extract ∴ mainly used as source of vitamins. contain carbohydrate, amino acid, growth factor.

4. Agar ∴ long chain of polysaccharide obtained from sea weeds: gelidium.

• contain Ca^{2+} , Cl, Mg, Fe, etc.

• used for the preparation of solid media.

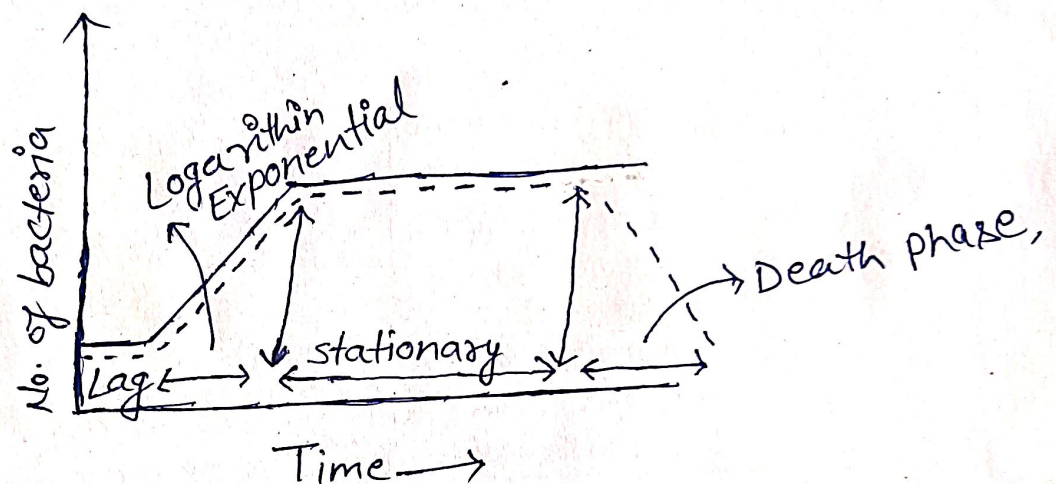
5. water ∴ - we use distilled water becoz normally tap water already microbes.

• use as solvent to dissolve molecules.

Bacteria Growth curve

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- * It is a curve that represent the different phase of bacterial growth. Shows.
- * In this curve, a graph plotted by counting the bacteria in a culture of definite time interval to show their growth stages.
- * The curve shows the following phase.



1. Log phase :- Immediately after inoculation there is no appreciable increase in number. There may be a increase in the size of the cells. This initial period is the time required for adaptation to the new environment and this lag phase, varies with species, nature of culture medium and temperature.
2. Log or exponential phase :- After the lag phase, the cell starts dividing and their number increase exponentially with time.
3. Stationary phase :- After a period of exponential growth, cell division stops due to depletion of nutrient and accumulation of toxic products. The viable count remains stationary as equilibrium exists b/w the dying cell and the newly formed cells.

phase of decline

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* this is the phase when the population decreased due to cell death.

Physical parameters or factors that affect the growth of bacteria

* many factors affect the generation time of the organism like temperature, oxygen, carbon dioxide, light, pH, moisture, salt concentration.

1. Nutrition: The principal constituent of the cell are water, protein, polysaccharides, lipids, nucleic acid and mucopeptides. For growth and multiplication of bacteria, the minimum nutritional requirement is water, a source of carbon, nitrogen and some inorganic salt. Some bacteria require certain organic compounds in minute quantities. These are known as growth factors or bacterial vitamins.

2. Oxygen: Depending on oxygen for growth and viability, bacteria are divided into aerobes and anaerobes. Aerobic bacteria require oxygen for growth. They may be obligate aerobes like *Vibrio cholera*, which will grow only in the presence of oxygen or facultative anaerobes which are ordinarily aerobic but can grow in the absence of oxygen. Most bacterial of medical importance are facultative anaerobes, anaerobes. Anaerobic bacteria, such as *Clostridia*, grow in the absence of oxygen and the obligate

anaerobes may even die on exposure to oxygen. (25)
microaerophilic bacteria are those that grow (27)
best in the presence of low oxygen.

3. Carbon Dioxide :- All bacteria require small amounts of carbon dioxide for growth. This requirement is usually met by the carbon dioxide present in the atmosphere. Some bacteria like *Brucella abortus* require much higher level of carbon dioxide.

4. Temperature :- Bacteria vary in their requirement of temperature for growth. The temp. at which growth occurs best is known as the optimum temp. bacteria which grow best at temp. of $25-40^{\circ}\text{C}$ are called mesophilic. Psychrophilic bacteria grow temp 20°C .

5. moisture and drying :- water is an essential ingredient of bacteria protoplasm and hence drying is lethal to cells. The effect of drying varies in different species.

6. Light :- Bacteria except phototropic species grow well in the dark. They are sensitive to Ultraviolet light and other radiations. culture die. if exposed to light.

7. pH :- Bacteria grow best at neutral or light slightly alkaline pH ($7.2-7.6$).

Preservation methods four pure cultures # (28)

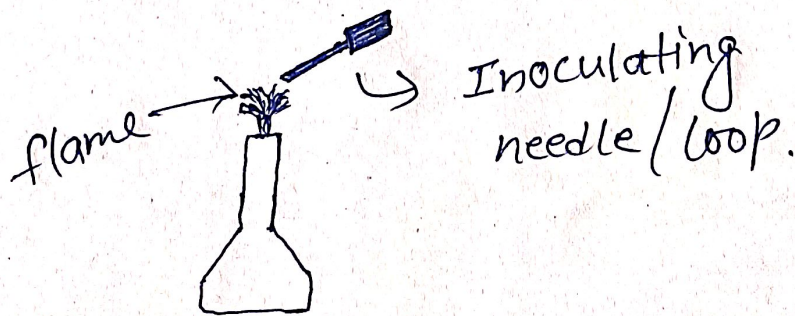
- * contains only a single species of bacteria.
- * After flask / test tube and pour into petri dish then closed in incubator for 2 or 3 day many bacteria are grown.

→ isolation is done by :-

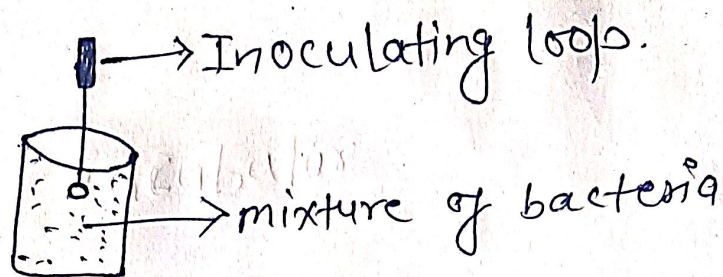
- Streak plate method
- spread plate technique.
- Pour plate method

(i) Streak plate method :-

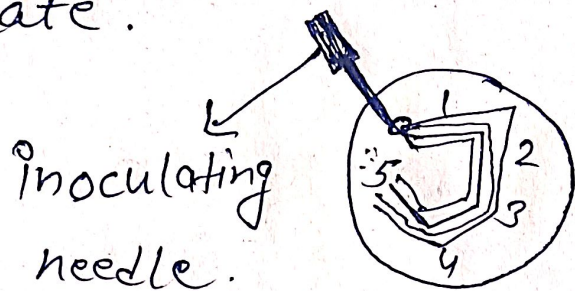
- * streaking is a process of spreading the microbial culture with an inoculating needle on the surface of the media.
- (i) But first we sterilize the inoculating needle by flame to make red hot and allow it to cool for 30 seconds or few second.



- (ii) Dip the loop into a sample containing a mixture of bacteria. the loop pick bacteria. (29)

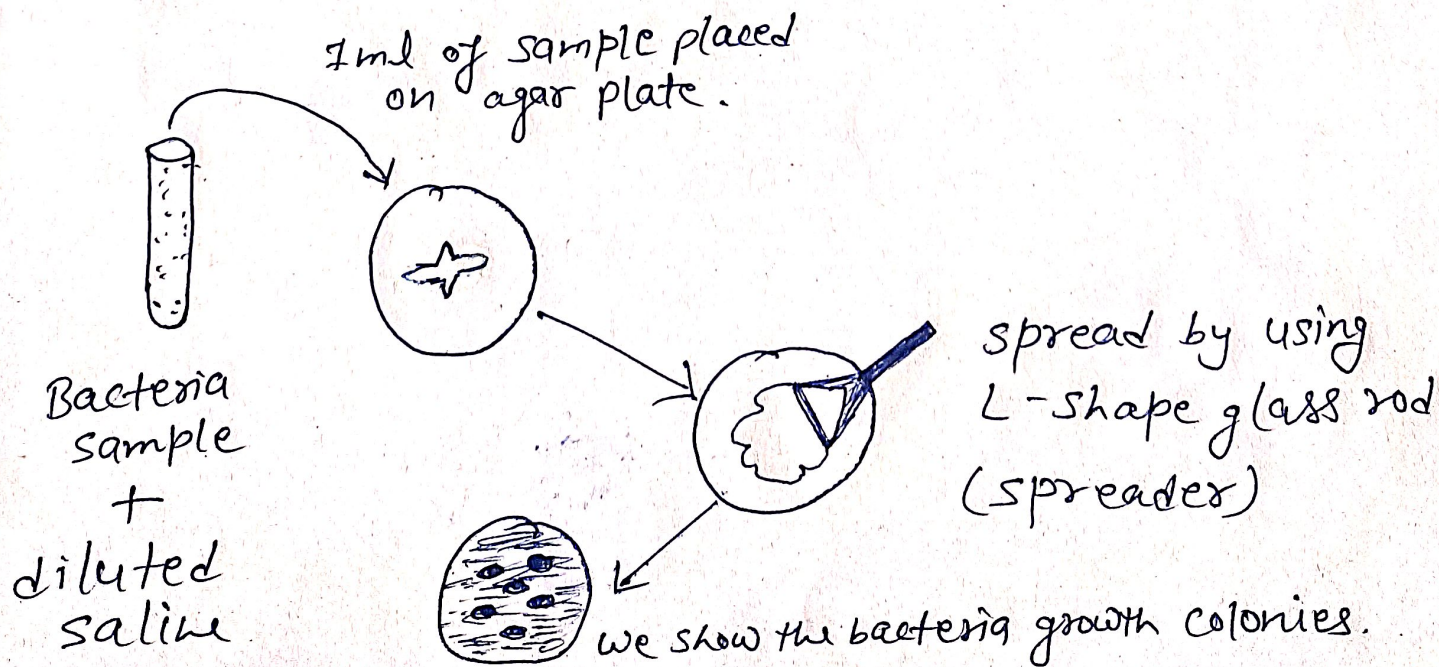


- (iii) Spread the bacteria on the surface of agar plate.



(ii) spread plate technique 00

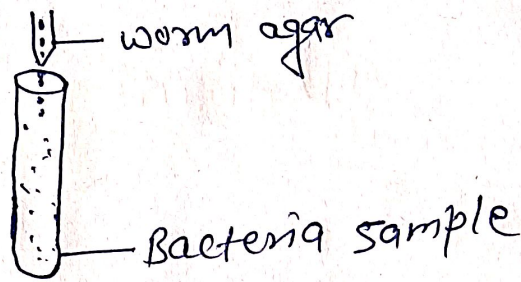
- * This is the best method to isolate the pure colonies.
- * In this technique, the culture media is not mixed with agar medium. Instead it mixed with normal diluted saline.



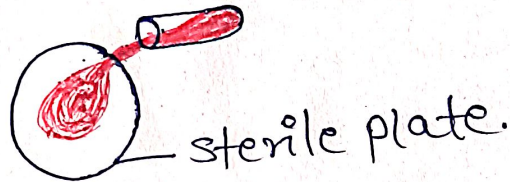
(iii) pour plate method

(30)

step 1 :- Bacteria sample mixed with warm agar ($45-50^{\circ}\text{C}$).



step 2 :- Sample poured on sterile plate.



step 3 :- sample swirled to mix, allowed to solidify.



step 4 :- Plate incubated until bacteria colonies grow.



Preservation of pure culture

(31)

1. culture Transfer
 - contamination doesn't mixed two culture.
 - genetic change
2. Refrigeration from 0°C to 5°C → 2 to 3 days.
3. low temp. freezing
 - Ultra low temp. freezer (-80°C).
 - liquid nitrogen (-196°C).
4. Lyophilization $\Rightarrow$
 - * freeze with dry ice and acetone
 - * use skin milk, glycerol or sucrose to protect cells.
5. paraffin oil → make a surface and the microbes not enter in culture media.

cultivation of Anaerobes

(32)

Growth of Anaerobes

* An anaerobe can be any organism that doesn't require oxygen for its survival or its growth.

* Types →
 ├─ facultative
 ├─ obligate anaerobes
 └─ microaerophiles.

→ facultative Anaerobes $\rightarrow$

* those anaerobes which generally grow in oxygen deficient environment but they can switch over to aerobic mode depending upon the situation.

→ Obligate Anaerobes $\rightarrow$

* Respire only in absence of oxygen and they died when oxygen exist on that medium.

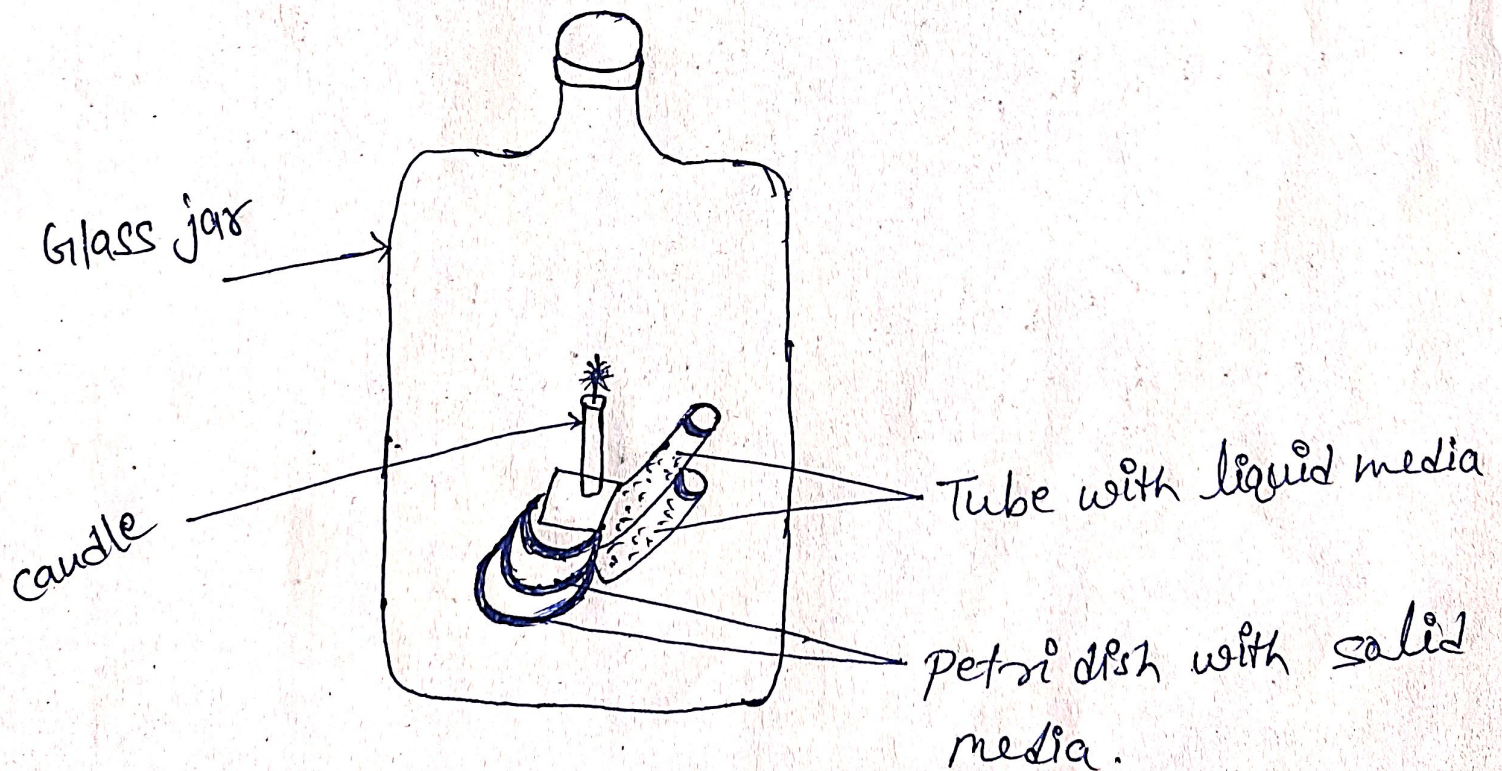
→ Microaerophiles $\rightarrow$

* Those anaerobic bacteria that grow under reduce oxygen and grow in presence of increased CO_2 level.

methods of cultivation of Anaerobes (33)

1. Candle jar method

- * It is one of the simple method for obtaining carbon dioxide environment for the growth of anaerobes.
- * Organisms like *Neisseria gonorrhoea*, *Streptococcus pneumoniae* etc. can be easily grown by this method.
- ⇒ Enclose the inoculated plates in an air tight jar with a burning candle, As the candle burns, the oxygen content is reduced leaving a carbon dioxide.
- ⇒ Candle itself act as an indicator as concn. of CO_2 inc. candle gets extinguished.

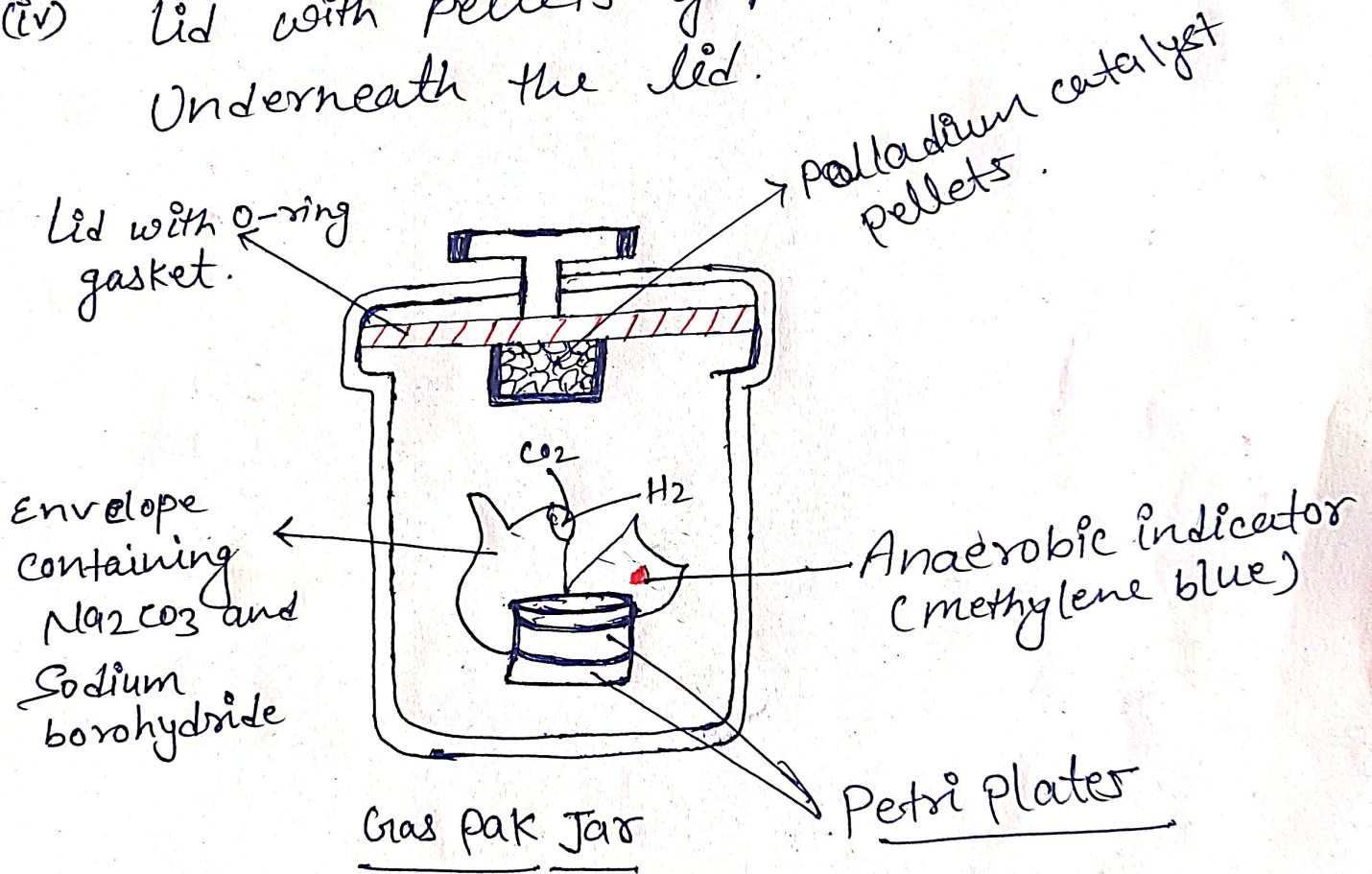


2. Gas pak jar method

(34)

→ It consists of —

- (i) Inoculated plate
- (ii) Packet of chemicals, containing sodium bicarbonate and sodium borohydride.
- (iii) methylene blue indicator.
- (iv) lid with pellets of palladium catalyst located underneath the lid.



Working → cultural plates are placed in jar
↓
packet of chemical moistened with H₂O
↓
H₂O reacts with chemical in packets produce H₂ & CO₂
↓
Hydrogen combines with free oxygen in chamber to produce oxygen.

Note :- In this way oxygen is removed completely from the chamber.

- CO₂ formation in anaerobic condition.
- methylene blue doesn't undergo any colour change.

Quantitative measurement of bacteria growth

- * After inoculation of bacteria into medium under suitable condition grow at very rapid rate.
- * for calculation of bacteria we do following procedure

- Total count → It measure all bacteria whether they living or died.
- Viable count → It measure only living bacteria count which produce colony on a suitable medium.

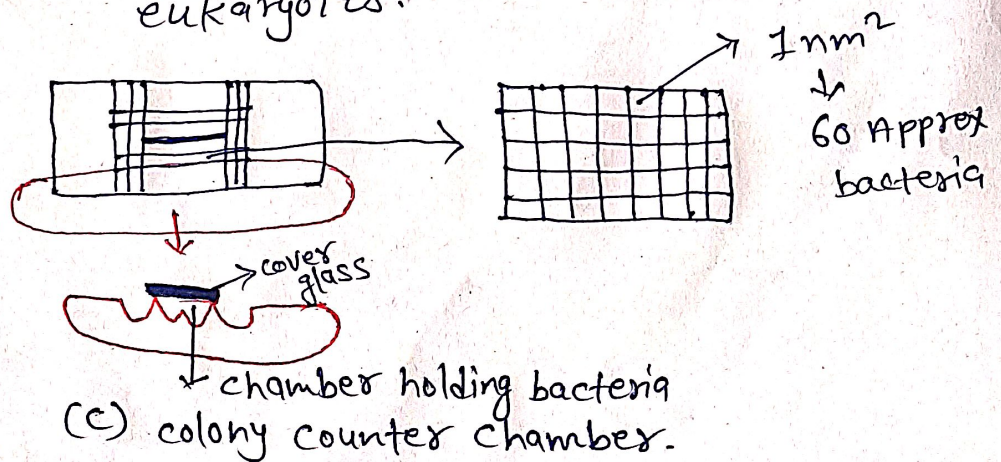
* Total count :-

→ Direct method :- total number of bacteria & its growth is calculated directly by counting the no. of colonies by using different techniques.

→ counting chamber method :-

Requirements → (a) Hemocytometer used for prokaryotes or eukaryotes.

(b) sample media

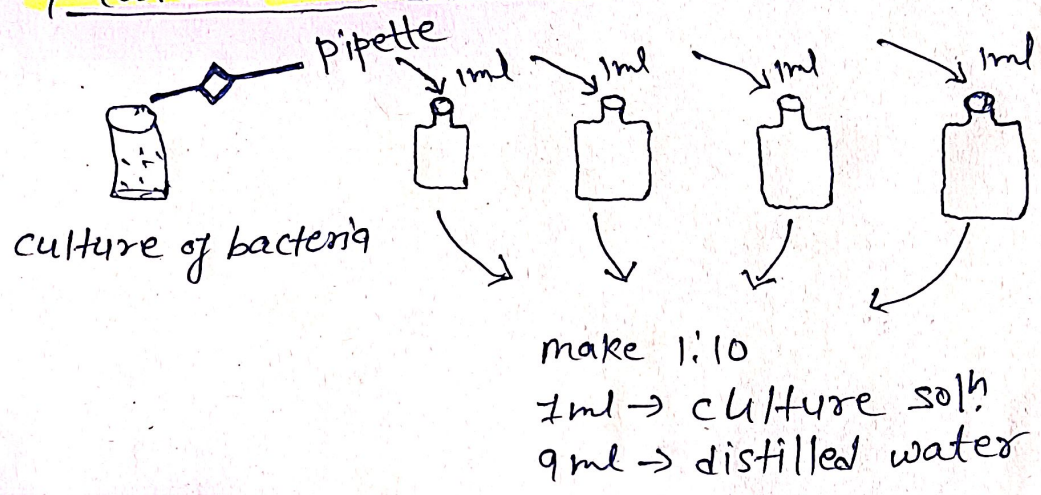


Advantage.

- * Easy or simple way to calculate.
- * quick method
- * inexpensive.

Viable count

→ Plate count method :- step - 1st



step - 2

mix the culture media in agar plate by rotating

Agar Plate

step - 3 :- Incubating them.

step - 4 :-

→ count plate containing 30-300 colonies select.



step - 5 :- calculate no. of colonies $\times$ dilution of counted on plate.

$\Rightarrow$ No. of bacteria / ml.

Microscopy

microscope is an optical instrument used to enlarge objects or microorganisms which can't be seen by our naked eyes.

* Types

Light microscope

→ use light to see object.

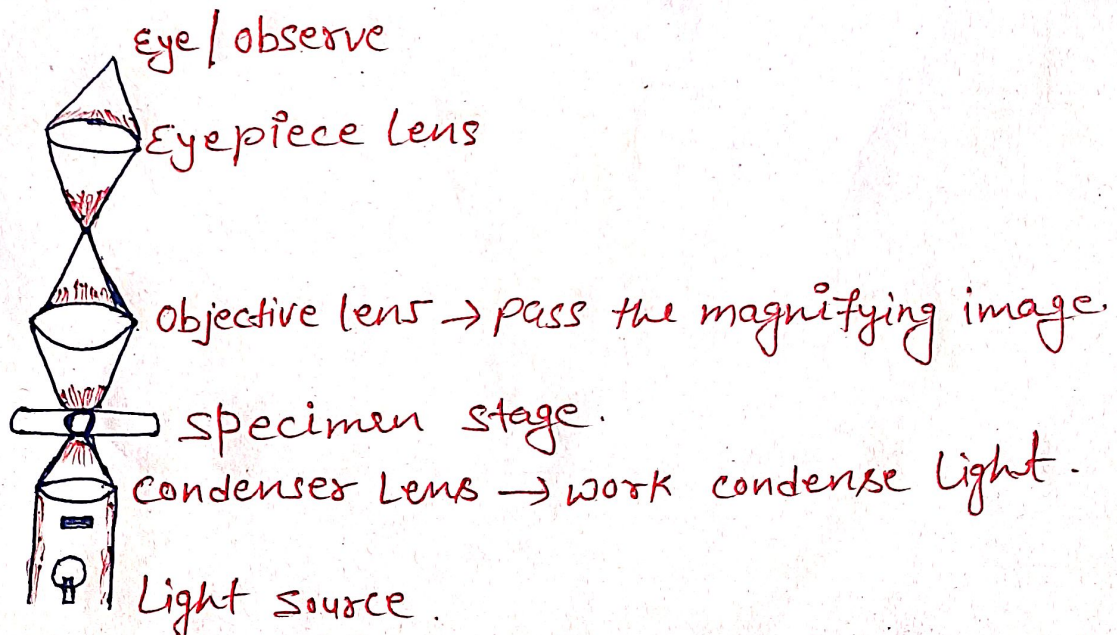
Electron microscope

→ use beam of e^- to see object.
⇒ electron microscopy

- (i) Bright field microscopy
- (ii) Dark field microscopy
- (iii) phase contrast microscopy

(i) Bright field microscopy :-

- * The name bright field is derived from the fact that the specimen is dark and contrasted by the surrounding bright viewing field.
- * Bright field microscopy is the most elementary form of microscope technique.



→ Advantage :-

- * Simple to use
- * Some specimen can be viewed without staining.

→ Disadvantage :-

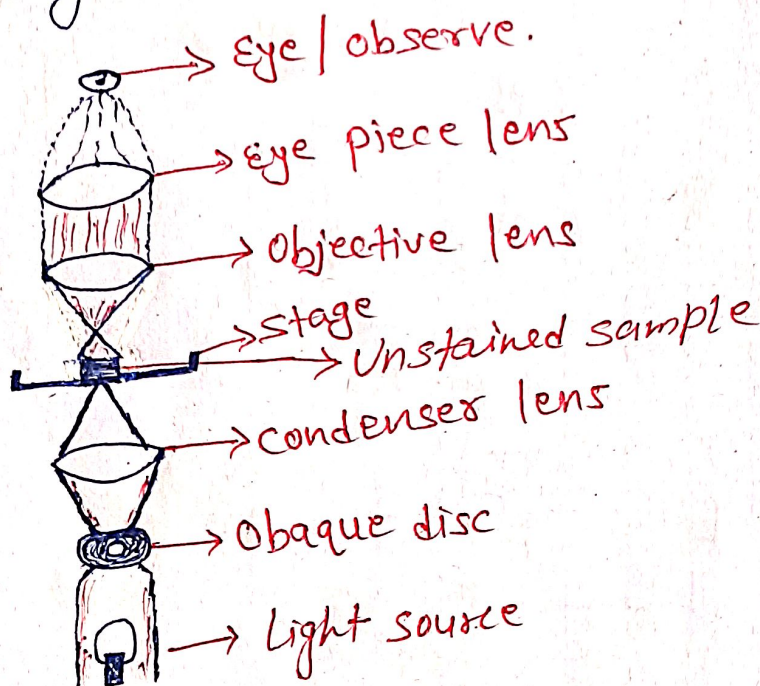
- * low contrast image form.

(ii) Dark field microscopy :-

- * used to see unstained samples.
- * Unstained sample appear brighter against dark background.

→ Principle :-

- * To see specimen in this microscopy, opaque disc is placed under condenser by object on slide reached to eyes.



→ Uses :-

- * Live detection
- * simple and effective
- * High Contrast

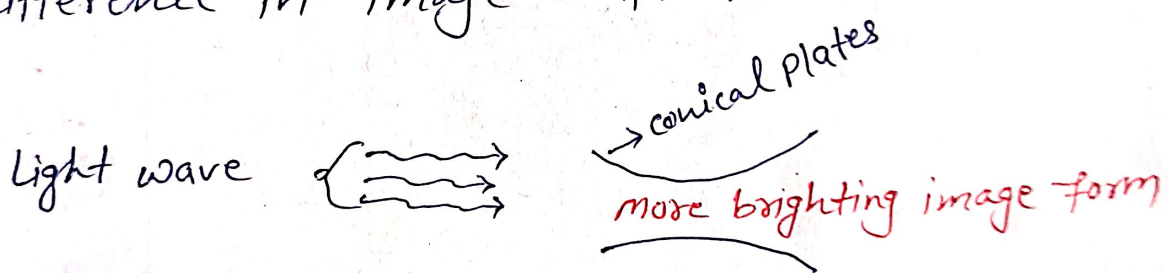
(ii) phase contrast microscopy :-

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- * It is contrast enhancing optical technique that utilize to produce high contrast images of transparent specimen such as living cells.
- * In this microscopy use conical phase plate which emerged the light in common / single phase.

→ Principle :-

- * When light pass through the cell, small phase shift occur which are invisible to human eyes.
- * In phase contrast microscopy, the phase shift are converted into amplitude which can observed as difference in image contrast.

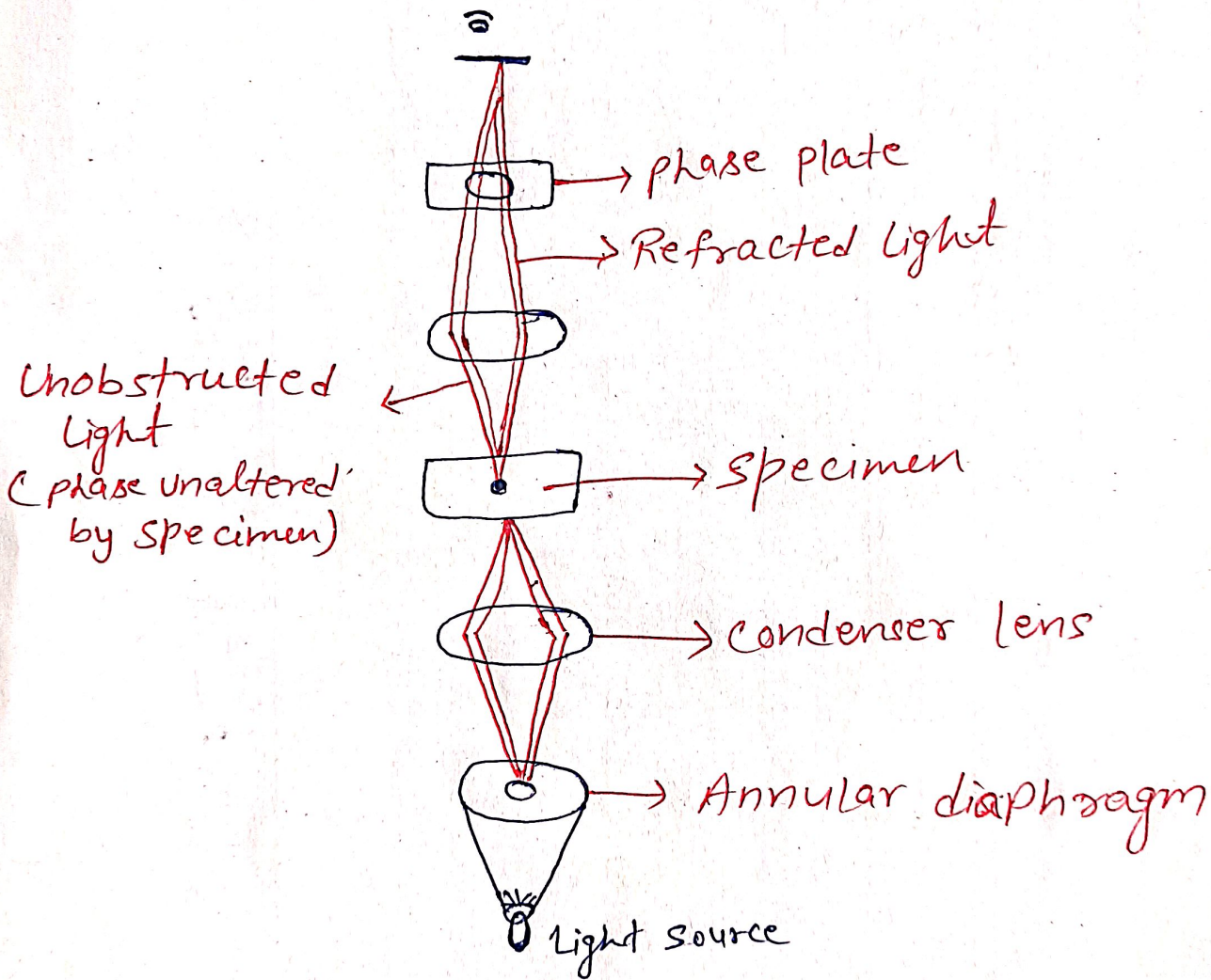


→ Application :-

- * To produce high contrast images of transparent specimen such as.
- * living cell
- * microorganism
- * fibres
- * study of cell division.

→ Advantages :-

- * Living cell → observed in natural state.
- * It make highly transparent object more visible.
- * Examine intracellular components.



Electron microscopy :-

- * Electron microscopy is a technique used to obtain high resolution images of biological and non biological specimen.
- * Used in biomedical Research also to find detail structure of tissues, cell organelles & macromolecules, complex.
- * Electron microscopy use magnetic field to form electron optical lens system.
- * Source of illumination $\rightarrow$ Beam of e^- use.
 $\hookrightarrow$ wavelength $\rightarrow$ electron 100,000 shorter than visible light photons.
- * **EM** $\Rightarrow$ Key information on the structure basis of cell & cell disease.
- * **Types** $\Rightarrow$
 - $\hookrightarrow$ Transmission electron microscopy (TEM).
 - $\hookrightarrow$ Scanning electron microscopy (SEM).
- (i) **TEM** $\circ$ To view thin specimen.
- (ii) **SEM** $\circ$ provide detail images of surface of cell.

