Pharmaceutical Microbiology :-

* Study of microorganism is K/a microbiology

- micro → minute, very small
- bio → living beings
- Logos → study

- father of microbiology → Antonie Von Leeuwenhoek
- Modern father of microbiology → Louis pasteur

* Branches of microbiology :→

① On the basis of application
② On the basic of study

- Virus – Virology
  Bacteria – Bacteriology
  fungus – Mycology
  Algae – Phycology
  Protozoans – Protozology

* The applied branch of microbiology

field		branch
→ Pharmacy	→	Pharmaceutical microbiology
→ Medicine	→	Medical microbiology
→ Agriculture	→	Agricultural microbiology
→ Mining	→	Geo microbiology
→ Ecology & environment	→	Environmental microbiology

- fermentation → Industrial microbiology and microbial biotechnology
- food → food microbiology
- Dairy → Dairy microbiology
- Ocean → Oceanography (marine microbiology)
- space → Exobiology

medical microbiology :- The study of the role of microbes in human illness includes the study of microbial pathogenesis and epidimiology and is related to the study disease pathology and immunology.

Pharmaceutical microbiology :- It is the part of Industrial microbiology i.e responsible for creative medicine as well as study of spoiler of medicine and there preservation.

Glycocalyx :→ layer of polysaccharide surrounds bacterial cell and protects from desiccation, also confers resistance to the pathogen from phagocytosis

Flagellum :→ Long hair like proteinaceous structure imparts motility to bacterium

Pilus :→ Short hair like proteinous structure imparts in gene transfer

Fimbrae :— Numerous very short havey proteinaceous structure helping bacterium in attachment to surface.

Cell wall :→ Gram positive bacterial cell wall contains peptidoglycan and Tachoic acid an Gram negative cell wall made up of

peptidoglycan, lipopolysaccharide, phospholipid and lipoprotein, provide protection and shape to bacterial cell.

Cell membrane :- Thin structure made up of protein and phospholipid enclose the cytoplasm, controls transport across the cell

Ribosomes :→ Granular small bodies made up of protein and rRNA involved in protein synthesis

Nucleoid :→ Genome region, Bacterial genome is made up of DNA : site of genetic code & controls heredity.

Plasmid :- Extra chromosomal DNA confers novel character to bacterium

Metachromatic Granules :→ Polyphosphate storage granules found in several bacteria

Mesosome :→ Inner folding of cell membrane provide extra space for enzymatic reacⁿ

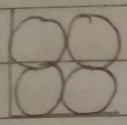
(I) spherical
(II) Rod shape / cylindrical
(III) Spiral

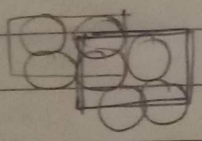
Spherical shape bacteria

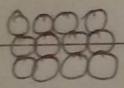
I- monococcus

II - OO Diplococci — eg — Diplococcus Pneumoniae

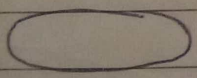
OOOOOO — streptococci — eg — streptococcus lactis

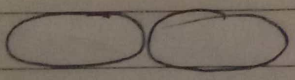
 — Tetrads

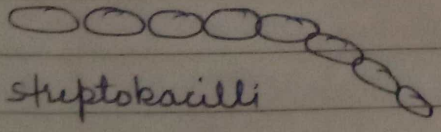
 — Sarcing
Sarcinae

 — staphylococci
→ Staphylococcus aevrous

(II) Rod shape / cylindrical


monobacillus (Lactic acid bacillus)


(moraxella bovis)
Diplobacilli


streptobacilli

Spiral shape bacteria :→ one or more twists they are never straight

Spirella :— Larger rigid rod with several curves or coils They are helical shape like a cork screw

eg:→ spirillum ruprom

Spirochetes :→ moves by and axial filament which is contain under the external flexible sheath. (sy

eg :— Treponema pallidum

Vibrio (,) — Comma shape bacteria :→

eg :→ Vibrio Cholera

Nutritional Requirement of bacterial growth

The growth of microbes depends upon adequate supply of suitable nutrients P^H, temp, and oxygen. All bacteria have three major nutritional need for growth.

i) A source of carbon for making cellular constituents.

ii) A source of Nitrogen for making protein

iii) A source of energy (ATP) in order to synthesize macromolecules and maintain essential chemical gradients across their membrane

Other essential chemical which { H_2, S, P, O_2, H_2O

trace element - Ca, Cu, K, Zn, Cl, mg, etc

- cation →
- anion → Cl^-, SO_4^{-2}

Organic growth factor :- ① Amino acid — ② purine and pyrimidines (3 type)

③ Vitamins

Physical Parameter :-

Some Physical Parameters are also required for the microorganism

Temp^r :-

pH :- (6.5 - 7.5)

Osmotic pressure :-

Temp^r :- On basis of temp^r range (microbes are classified into three groups)

① Thermophiles — hot loving — (55° - 85°) and ab

② Psychrophiles — cold loving — below 5°C

③ Mesophiles — ~~mesophile~~ moderate temp^r loving — 30° - 45°C

pH :- depending and optimum pH Value of microbes they can be classified as —

Acedophiles :- The microbes have optimum pH range in between 1.0 to 6.5

Neutrophiles :→ The microbes have optimum pH range in between 6.9 - 7.4

Alkalophiles :- The microbes have optimum pH range in between 7.5 - 14

Osmotic pressure :→ Is osmotic pressure [same pressure inside the bacterial cell]

Raw material for culture media :→

- water :- Generally tap water is used but it should have no mineral content.

Cupper containing distill water should not be used preparation of culture media

- Distill water is used for culture media

Sodium chloride (NaCl) and other electrolytes :→ are required for maintain the osmotic pressure

Beaf extract / meat extract :→

It is prepare from fresh meat

It is Yellowish brown in colour having meat like odour and taste

- It having organic micro nutrients, proteins and carbohydrates —

MALT extract :→ It is Brownish yellow colour prepared from sprouted grain prepare ~~from~~ aqueous extract of sprouted grain and dry at low temp it preserve with carbohydrate and Nitrogen it having various growth factors.

Peptone :→ It is water soluble compound obtained from lean meat (having low fat contain / skinless meat), Soya flour, heart muscle, casein.
It is an important source of nitrogen, phosphate, potassium, magnesium etc

Blood :→ may be collected from horse rabbit, sheep and some time from man.

Serum :- Plasma - fibrinogen
Serum may be prepared from defibrinated blood it is sterilized by membrane filter.
- It may be store at temp 3-5 C in the refrigerator

Caseine :→ It consist of amino acid obtain of by hydrolysis of milk protein caseine

Yeast extract :→ It is prepare from cells of saccromycetes it is source of Vitamin B_{12} amino acid and certain element

AGAR :→ Agar is a long chain polysaccharide. It is a mucilagenous substance extract from Gelidium Corneum

It is used as a solidifying agent

- Soluble in hot water but insoluble in cold water
- Agar in molten form at temp^{r} above 40° C

Culture media :→

Media is ~~an RBC~~ an artificial ~~bacteria~~ mixture of various nutrient in appropriate concentration,

- Media is prepared by considering the biochemical requirement of microbes
- Media are used in the laboratory for the cultivation of bacteria. Media are must supply all of the neccesary nutrient required for the cellular growth and maintaince of the organism.

Characteristic of an Ideal media :-

- It should give satisfactory growth from single innaculum
- It should give rapid growth
- It should be reasonabny cheape
- It should be reproducible

Types of culture media :→

Media are following type on the basic physical State

- liquid
- Solid
- Semi-Solid

liquid :- media have liq consistency K/a nutrient broth

Solid :- If Solidifying agent is added media it confirms gives solid consistency to media

Semi-Solid :- If Agar is added in low Concentration it than gives Semi-Solid Consistency to media

Media based on chemical compound :→

I- Synthetic or difined media
II - Non-Synthetic
III - Enrichment
IV - Selective
V - Differential
VI - Aerobic
VII - Anarobic

Synthetic or difined media :-

These media are prepared by all chemical substance and the exact composition of medium is known

- It provides only the exact nutrient to organism for growth

Non synthetic :→

Not exact composition of the chemical for the media

- It is not difiend
- This is the common nutrient medium which is provide to all type of microorganism
- Its having Beaf extract peptone, NaCl water and agar used at a solidifying agent

Enrichment media :→

i) Lag phase :→

There is no bacterial growth in this phase

- When bacteria are inoculate into fresh medium multiplication usually does not begin immediately.
- The period b/w innoculation and beginning of multiplication is K/a lag phase
- During this period the organisms adapt themselves to growth in fresh medium and increase in size as well as metabolic activity. therefore lag phase is regarded as a period not of rest but of intense metabolism activity

ii) Log phase or exponential growth phase :→

- During phase the bacteria are multiplying at their maximum rate and their number increases exponentially with time
- The time required for are bacterial division during exponentially with time
- The time required for are bacterial division during this phase in K/a Generation time
- All the number of organism present in each generation period is almost twice that in previous period
- Exponential growth phase is of limited duration because of -

- exhaustion of nutrients / lack of
- accumulation of toxic metabolic end product
- Rise in cell density
- Change in pH
- Decrease in oxygen level (incase of Aerobic organism)

iii) Stationary phase :-

Due to the following reason

- exhaustion of nutrients
- accumulation of toxic metabolic end product
- Rise in cell density
- Change in pH
- Decrease in oxygen level

- The exponential growth phase slow down and bacterial population enters the stationary phase in which the number of Viable cells remains Constant
- There is almost b/w the bacterial reproduction and bacterial death

iv) Decline phase (death phase)

In this phase the rate of death exceed the rate of reproduction and the number of Viable cells declines / decrease

Isolation and preperation of pure culture

Culture - Culture is the art of cultivating microorganism !!

Mixed culture - Mixed culture is a culture containing more than one microorganism

Pure culture :- Pure culture mainly consist of single species of organism which is usually derived from mixed cultures

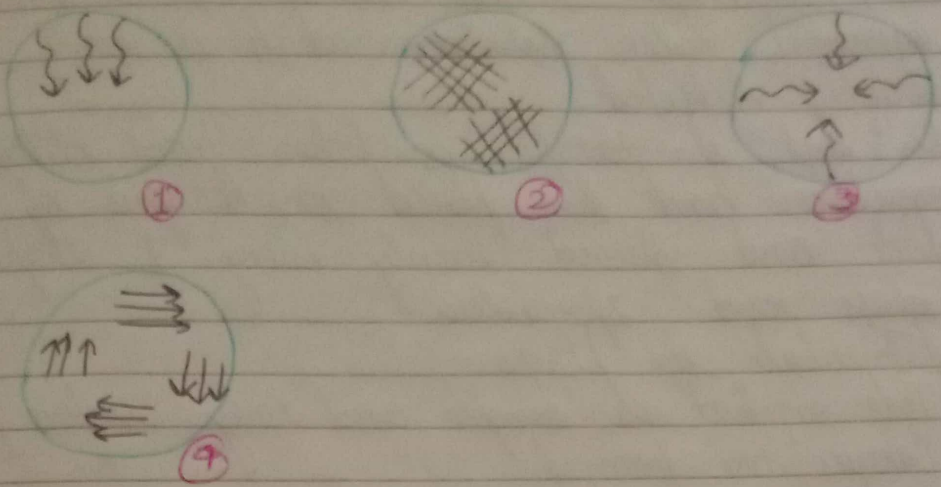
Isolation methods of pure culture -

Streak plate method
Pour plate method
Spread plate method
Roll plate Method

Streak Plate Method

Streaking is the process of spreading the microbial culture using inoculation loop on the surface of the media
Sterilised the inoculation loop by flame to make it Red hot
Allow it to cool for 30 seconds.
The sample is spread in such a way to get series of dilution
Purpose is to get seperate colonies

Method of Streaking



Pour plate Method

Bacterial culture and liquid culture medium are mixed together.
After mixing the medium containing bacterial culture is poured into Sterilized petri dish.
Then incubate for desired temp and time.
In this method microorganisms are trapped beneath the surface of medium which make colonies counting difficult.

Roll tube Method

In this method exposure to air is avoided by displacing air in culture vessel with an Oxygen free gas such as CO_2, Nitrogen and H_2

Spread plate Method

In this technique Bacterial culture is not mixed with agar medium instead it is mixed with normal Saline solution which is then serially diluted.

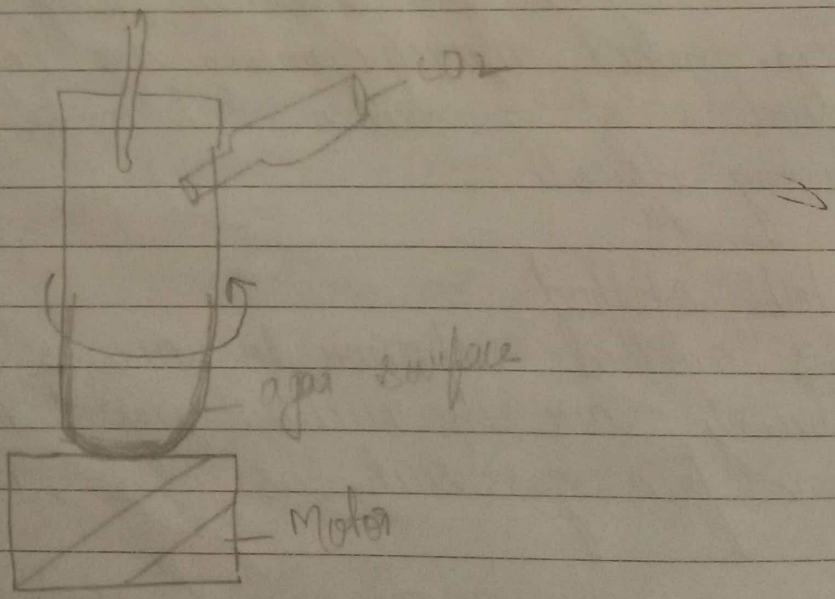
0.1 ml of sample is taken from diluted mixture and then placed on surface of agar plate and spread evenly using L shaped glass rod spreader.

Incubate the plates

This method is simple and only surface colonies are formed

Roll tube Method

In this method exposure to air is avoided by displacing air in culture vessel and oxygen free gas such as CO_2, N_2, H_2



Preservation of pure culture

To maintain pure culture for extended period in viable condition without any genetic change is termed as preservation

(i) Periodic transfer to fresh medium
(ii) Storage at low temp.
(iii) Storage of sterile soiled
(iv) Preservation by overlapping with mineral oil
(v) Lyophilisation or freeze dryer

Periodic transfer to fresh medium

Bacterial strain can be maintained by periodically preparing a fresh culture from previously stock culture

Culture medium, storage temperature and time interval at which transfer are made vary with the species.

Temperature of and type of medium choosen should afford a slow rather than rapid growth so that time interval b/w transfer can be as long as possible

Storage at low temp. (Refrigeration)

Pure culture can be successfully stored at 0-4°C either at refrigerator or in cold rooms.

This method is applied for short duration i.e. 2-3 week for bacteria and 3-4 mont for Fungi

Cryo Preservation (Freezing in liq. Nitrogen at -1

This method also helps in survival of pure culture for long storage time.
Stabilising agent such as glycerol or dimethylsulphoxide (DMSO) also prevent cell damage due to formation of crystal and promote cell survival.

Storage in Sterile Soil

Storage in sterile soil fall into two groups.
(i) Sterile soil infested with small amount of inoculum, immediately dried and stored in refrigerator
(ii) soil infested with M.O, then incubate to grow and this Mycelium for 2nd generation is preserved → 2nd group is used for fungi & by this method fungi can be preserved for 4-5 y. Bacteria can also be preserved for several years.

Preservation by Overline with mineral oil

This method is simple and economical
In this method liq. paraffin (mineral oil) poured over slant of culture and stored upright at room temperature.
The layer of paraffin ensure anaerobic condition and prevent dehydration of medium M.O. remains at dormant stage can be stored for years.

Lyophilisation or Freeze drying

Freeze drying is a stabilising process in which substance is first frozen and then quantity of solvent is reduced first by sublimation (Primary drying) and desorption (Sec. drying)

I. Solidification
II. Sublimation
III. Desorption

This process combined freezing & dehydration
This method reduces risk of intracellular ice crystallisation
Cell viability can be maintained can long as 30 yrs.

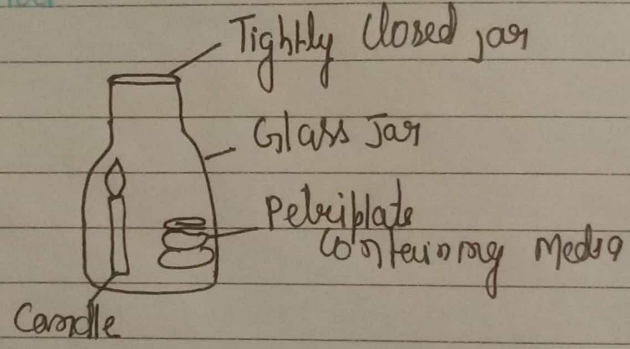
Cultivation of Anaerobes

Anaerobes :- Those microorganism or bacteria which do not requires Oxygen for its survival or growth are termed as Anaerobes

For cultivation we have to create in which oxygen is not present
Two method are generally used :-

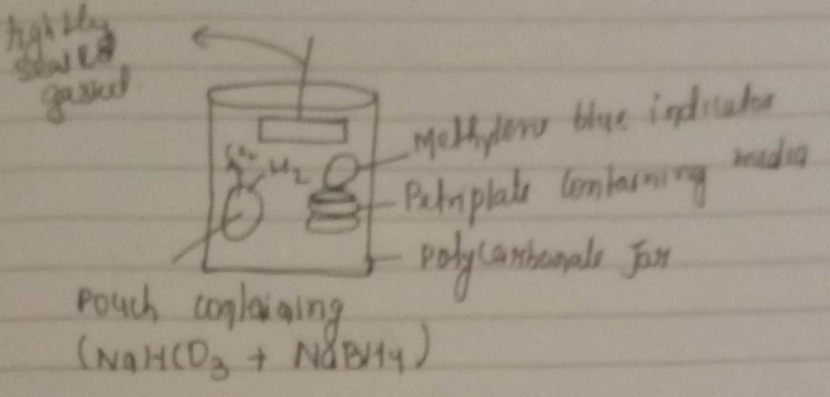
- Candle jar Method
- Gas pak Jar Method

Candle jar Method :-



In this method petriplate containing culture media is placed inside closed jar with the burning candle
Candle require Oxygen for burning So as the candle will burn the oxygen will reduced and CO_2 conc^{n} will increased
Candle act as an indicator when it get extinguished this will show that oxygen empty Jar is ready for growth of Anaerobes.

Gas pak Jar Method :-



In this method inoculated plates containing media is placed inside the polycarbonate jar with gas generator pouch and an indicator strip
The jar is then sealed completely
The chemical inside pouch react to produce CO_2 & H_2
The CO_2 will replace oxygen and create anaerobic environment