* Introduction of Microbiology

* Microbiology in the study of living organism of microscopic size.

* The word Microbiology is derived from three greek words :-
  i) Mikros (small)
  ii) Bios (life)
  iii) Logos (study)
  Therefore Microbiology deals with the study of microorganism.

* It also includes the study of abilities of micro organisms to make physical and chemical changes in our environment and their reaction to physical and chemical reagent.

⇒ Microbiology :- Example* :-

i) Protozoa → Protology
ii) Virus → virology
iii) (Connecting link b/w living and non-living matter)
iii) Fungi → Mycology
(Heterotrophic eukaryotic organism)

iv) Alage → Phycology
(autotrophic eukaryotic organism)

v) Bacteria → Bacteriology
(It deals with the study of bacteria)

* Bacteria have profound influence on human being, including health, industry, agriculture, e.t.c.

* Medical Microbiology :- The branch that deals with study of pathogenic microbes; their life cycle, pysiology, genetic.

* Agriculture Microbiology :- It deals with the study of robes of microbes in agriculture, both harmful and useful.

* Industrial Microbiology :-

It deals with the study of microbes in industry many microbes Produce medicinal Product like antibiotics and medicin vaccine. fermented beaverages industrial chemicaly production of protein and harmones by genetically engineered microorganisms.

* Food Microbiology :-

In this branch various aspects such as food processing, food preservation, canning pasteurization of Milk, study of food born microbial disease and their presention is studied.

* Water Microbiology :-
→ Microbial examination of water.
→ Study of Microbes present in water.

* Aero Microbiology :-

→ Study of Microbes Present in air.

* Environmental Microbiology :-

Study of Microbes Present in environment.

* Geochemical Microbiology :-

Study of microbes present in soil, coal, mineral and gas formation.

* Exomicrobiology :-
Study of life in outer space.

* Pathogen :-
disease causing microbes.

* Advantages of Microbes :-

→ Used in Fermentation.
a) Yoghurt Production.
b) Acetic acid Production.
c) Bread Making.

d) Wine Production / Alchohal Production.
e) Pickel Making
f) Butter Making
g) Cheese Making
h) Antibiotic Production

* In Agriculture :-

a) used as fertiliser
b) used as bio-fertiliser.
c) used as pesticides.
E.g.- cry gene of Bacillus thuringenesis was used to produce Bt cotton. The cry gene produce a Protein which on cleavage yield insectisidal crystal protein (ICP) which acts on gastro-intestinal thus leads to the death of larvae by starvation.

* Vaccine Production :-
E.g. → Poliovirus. Smallpox virus.

* Used as food :-

a) Mushroom
b) Algae
c) Single-cell protein (Spirillum)

* Pollution indication :-
E.g. → Lichen

* Gene transfer
* Enzyme Production
* Soyabean Production
* In cancer Treatment.

★. Disadvantages of Microbes:-

* It causes disease
* Spoils food
* Pollute water
* Storehouse of Pathogen.
* Produce greenhouse gases.
* Destroy woodpiller.

=> E. coli is 500 times smaller than average Plant and Animal cell.

=> The rate of Microorganisms is very high under normal laboratory.
Example → Some bacteria double their number in every 20 minutes.

☆ History of Microbiology :-

* In the Poem "De rerum nature" Lurretious (967 - 55 BC) mentioned the existence of "seeds of disease".

* In 30th century Roger Bacon (1220 - 1292) suggest that disease are produced by Invisible creature.

* This opinion was supported by Giro Lamu fracastro verona (1483-1553) and Anton von Plencing in 1762. But these people had no proof.

* In 1558 a monk Athanasius Kicher. reffered to "worms" invisible to naked eye in decaying bodies, meat, Milk and cliorrheal secretion.

* Kicher was the first Person to recognise the significance of bacteria and other microbes in diseases but his discription lacked accuracy.

☆ Antony Von Leuwenhook (1632-1723)

i) A Dutch cloth merchant in Holland.
ii) His hobby is lens grinding and microscope making.
iii) During his lifetime he made more than 250 microscopes. consisting of home ground lenses

mounted in brass and silver.

iv) The Microscope of Leuwenhoek could magnify objects to 200-300 times.

v) on the series of the ~~series of~~ Royal Society of London he described the variety of microorganisms such as protozoa, Algae, yeast and bacteria.

vi) In 1683 he described and sketched different form of Animalcules. i.e. rod, spheres and spiral shapes.

vii) This was the first recorded observation of bacteria.

☆ Luis Pasteur (1820 - 1895)

i) A Professor of organic chemistry in France

ii) Pasteur was named as "father of Microbiology"

iii) The Pasteur institute was built for him in Paris by Public Support.

iv) His main contribution are:
a) He seprate the crystal of tartric acid
b) He coined the term of "fermentation".

=> Fermentation :-

→ According to Pasteur. fermentation occurs in the presence of oxygen which Produces alchohal and acid.

→ Pasteur said that the microbes can groco in aerobic as well as anaerobic condition.

→ He suggested that the undesirable type of microbes can be destroyed by heating upto 68.2°C for half an hour. This Process is called Pasteurization.

⇒ Pasteurization :-

→ In this Process decommedity is heated at 68.2°C for 62 minute It is called as slow heat longer duration pasteurization.

→ It is used in diary industry.

→ In this process the decommedity is heated at 30°C for 3 minutes. this is called as strong heat short duration pasteurization it is use in fruit juice.

c) He discovered the theory of Spontaneous generation.

→ He Performed the same type of experiment as spallengani

→ Pasteur allowed the air to enter into the flask of sterile broth.

→ He Performed experiments with 2 flasks, one with straight neck and other S-shaped neck.

→ Flasks with a straight neck allowed both air and microorgans to enter whereas the other flask with S-Shaped neck allowed only air to enter but not micro organisms.

→ The broth is straight neck become contaminated with micro organisms but the broth in the flask with an S-shaped neck didn't become Contaminated.

→ Therefore Luis Pasteur showed that even though the Air could get into the flask but the broth didn't produce micro organisms.

→ Pasteur also showed that Air contain microbes and a number of microorganisms in the Atmosphere vary from Place to Place. He also prove this by an experiment.

☆ Discovery of Aerobic and Anaerobic bacteria:-

→ Scientist in the middle of 15th century assumed that microbes also require oxygen.

→ During the study on butanic acid fermentation Pasteur discovered that microbes can survive in the absense of oxygen.

→ Microscopic observation of the motile butanic acid bacteria showed that the motility of the bacteria stopped when they came in the contact of air.

→ He also found that many other microorganisms including yeast could grow either in the presence or in the absence of oxygen. These microbes are termed as facultative anaerobes

d) Work on Silkworm Disease

→ In 1965 Pasteur was asked to find the cause of Peprine, a disease affecting silkworm.

→ He demonstrated that the disease was caused by Protozoa.

e) Work on protection against infection.

→ In 1880 Pasteur isolated bacteria responcible for cholera and view it in a pure culture

→ Pasteur also demonstrated that the anthrax

disease in sheep is caused by rod-shaped bacteria (Bacillus) and culture them.

→ He also showed that innoculation of weeked microbes to Animal provide them immunity thus he has discovered the Anthrax vaccine.

→ On the principle Pasteur also developed vaccine for Rabies, a dengerous human disease caused due to rabies dog bite

☆ Joseph Lister (1827-1912)

i) He was an English surgeon in London.

ii) Prevention of wound infection-

→ He talked about wound infection and started studying the reason for it.

→ He observed a similarity between a wound infection and fermentation described by Pasteur.

iii) Pure culture technique :-

→ He demonstrated the Procedure of isolation of Microorganisms from curd.

→ In 1978, Lister for the first time obtained pure culture of bacteria by using "Serial dilution techniq

in liquid medium.

→ He demonstrated plate method for the culture of bacteria.

☆ Robert Koch (1843-1910)

I) Robert koch, a german medical practicioner switched over the microscopic studies which engaged his full time.

II) His main contribution are:

a) Germ theory of disease.

→ He showed that microbes cause disease in animals by studying the Anthrax disease in cattle. Koch had found rod shaped organisms in blood of diseased animals

→ The test if these organisms were the causative agent of disease. he inject the bacteria in the healthy mice with the blood from diseased Animal.

→ He found that the disease was transmitted to the mice which is dead.

→ He took the blood of the dead mice and injected it into healthy Mice and again

The mice dead.

→ Koch repeated this experiment twenty time and each time healthy Mice dead.

b) Study of aqueous houmor from the eye of a cow -

→ Koch cultured aqueous houmor from the eye of a cow. by injecting these organism into healthy animals. he was able to reproduce the disease.

iii) From These studies he can able to lay down Certain basic criteria for the Indetification of microorganisms as positive agent of disease

The criteria are known as Koch postulate

Koch's Postulate :-

i) An organism has disease because of causitive organism.

ii) The causative ~~disease~~ (organism can be Isolated from the diseased animal.

iii) when the causative organism re-innoculated (injected) in a healthy animal it cause the same disease.

iv) The second animal also shows the presence of same causative organism which was present im the first organism.

c) Discovery of Tuberculosis Bacilli:-

Koch discovered the Tuberclosis Bacilli, a disease caused by microbacterium tuberculosis.

d) Introduced the method for making smears of bacteria:-

Koch introduced the method for making smears of bacteria on glass slide and staining them with aniene dies to observe them more clearly under the microscope.

e) Plate methods for isolating Pure culture

Koch accidently observe that a slice of potato had colonies of bacteria distinct from one another.

☆ Alexender Flaming :-

→ In 1927, Alexender flaming a microbiologist working in a St. merry Hospital in London discovered ~~Flam~~ penicillin. The first wonder drug.

→ Flamming in his laboratory was conducting experiments in the search of new anti-bacterial agents against wound infection.

→ In the course of experiment, he observed a plate culture of staphylococcus Dareus was contaminated by a blue-green colour mould.

→ The area around the edges of the mould colony was clear that is the growth of staphylococcus cell near the mould was inhibited or was killed by the mould.

→ The ~~inclo~~ introducing mould was then identified as a commonly blue-green mould Penicillium notatum.

Note :

☆ About the history of Microbiology :-

* Antony von Leeuwenhook discovered bacteria from stored drain water and in scum and called them "wild animalcules".

* He is known as discoverer of microbial world.

* "Ehrenberg" give the term 'Bacteria'.

* 'Nageli' Placed the bacteria in 'Schizomycetes' called 'fission fungi'.

* 'Luis Pasteur' Proposed the 'germ theory of disease'

* He discovered bacteria used in chicken cholera, invented 'Anti-rabies vaccine'.

* He gave the term 'Micro organism'.

* He is known as the 'father of Microbiology' and 'father of Sterilization Technique'.

* Robert Koch gave Kochi Postulates.

* Joseph Lister developed the technique of Aspetic culture.

* D.A. Bergey gave the classification of Bacteria in the manual of determinative bacteriology.

* C.E. Dillot used the term 'Microbes for Animalcules'.

Classification :-

CLASSIFICATION:

* Aristotle was earliest scientist who made an attempt scientifically for classification and use of the plants and Animals for deviding into various group.

→ System of classification

i) 2-kingdom classification-

Carlous Linnoeus gave this system and devided all organism into 2 kingdoms.
Plantae and Animalia.

ii) 3-kingdom classification-

This system of classification was Proposed of Earnest Haeckel. He seprated out all unicellulars into a seprate kingdom Protista (Fungi, Protozoa, algae, bacteria and Slime mould). Thus he Proposed 3-kingdom namely.
Protista, plantae and Animalia.

iii) 4-kingdom classification: Copeland (1956) gave 4 kingdom classification including Monera in it. Thus he proposed 4-kingdom namely.
Monera, Protista, Plantae and Animalia.

iv) 5-kingdom classification-

It was Proposed by R.H. Whittaker, the five kingdom are Monera, Protista Fungi, Plantae and Anamalia.

v) 6- Kingdom classification-

It was given by Carl woese. The six kingdoms are Eubacteria Archaebacteria, Protista, Fungi, Plantae and Animalia.

He seprated the archbacteria from Eubacteria on the basis of major difference such as:

→ Presence of peptidoglycan in the cell wall and the occurance of branched chain lipids (a monolayer instead of the phospholipid bilayer in the membrane

→ based on the sequence of 16 S rRNA Woese found that the 6-kingdoms naturally cluster into 3 main categories. He called these three categories as Domains. These Domains are Bacteria, Archaea and Eukarya.

All these three Domains are evolve from a common ancector called Progenote

☆ Archaebacteria ⇒ An oldest living fossils.

→ Cell wall lack muramic acid and Peptidoglycan

→ Contains Phytayl side groups (branched chain lipid)

→ Archaebacteria are of following types:

a) Methanogens :-

These are obligate anaerobic form of gram negative bacteria that produce methane gas. from CO_2 or formic acid.

→ These bacteria found in the rumen (First Part of stomach) of cattle and in marshy area.

→ In biogas Plants they produce methane gas.

→ Cell wall contains protein. (Example → Methanospirillum and methano coccus)

→ Non -cellulosic polysaccharide.
(Example → Methanosarcina)

→ Pseudomurin (Ex → methano bacteria) in which N-acetyl Talosamiuronic acid (NAT) is present instead of (NAM)

b) Halophiles :-

→ These are gram negative, obligate anaerobic with coccoid form of bacteria.

→ Habitat - tidal pool, salt ponds, brines salted fish, salted hides.

→ Halobacteria can grow well in culture medium

containing as high as 25% - 35% of NaCl.

→ In the presence of sunlight with the help of purple pigment. Synthesis ATP.

C) Thermophiles :- Thermoacidophiles :-

→ These are aerobic form and gram negative bacteria found in Hot-Sulphur springs.

→ At a temperature of about 80°C they oxidise sulphur to sulphuric acid

→ They can survive at a highly acidic medium (pH = 2)

→ under aerobic condition, they reduce sulphur to hydrogen sulphide and under aerobic condition they oxidise sulphur to sulphur dioxide.

→ They are Faculative anarobes and are chemosynthetic in Nature.

→ Example → Thermoplasma and Thermoproteo
ph → 2; temp. → 100°C

★ Size, Shape and arrangement of bacterial cell

* Bacterial size vary from 0.2 Hm to 2 μm in diameter and 0.5 to 5 Hm in length

* largest bacteria -

- Thiomargarita namibinisis
- Epulopicium

} 600 μm in length and 75 Hm in diameter.

* Smallest bacteria -
- Mycoplasma <0.3 Hm in diameter>

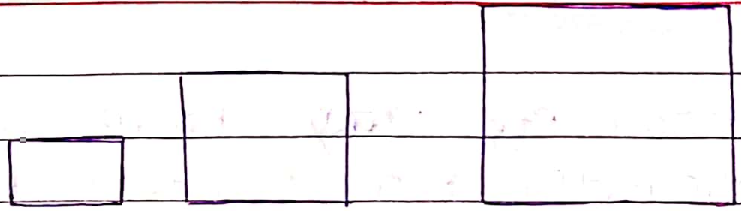
* mostly bacteria are small in size.

* They have large surface area to volume ratio. It means that no internal part of the cell is very far from the surface and the nutrient and can easily and quickly reach to all part of the cell.

* It also allow rapid uptake and intracellular distribution of Nutrients and excretion of waste materials.

* at low surface area to volume ratio. the diffusion of nutrients and waste product across the cell membrane limit the rate at which microbial metabolism occurs making the cell less evolutionary fit.

*



Surface area	6 mm	24 mm	96 mm
volume	1 mm^3	8 mm^3	64 mm^3
Surface ratio / volume	$\frac{6}{1}$	$\frac{24}{8} = \frac{3}{1}$	$\frac{1.5}{1}$

* Shape

i) Coccus (pl. cocci) => Spherical

a) micrococcus → Single Sphere
Example → micrococcus flagyy

b) Diplococcus → Sphere in pairs
Example → Diplococcus pneumoniae

c) Streptococcus → cells remain attached to form a chain.
Example → Streptococcus lactis.

d) Stephylo coccus → Irregular bundle of cell or grape-like cluster
example → Stephylococcus aureus

e) Sercinae → 3-dimensional geometrical

figure like cube

example - sercina.

ii) Bacillus <Pl. bacilli> => rod shaped / sigarette-like with rounded and Blunt end

=> most common shape
=> Can be motile and non-motile.

a) Monobacillus → Single

b) Diplobacillus → Group of two

c) streptobacillus → Found in chain

d) Trichomes → when the cells of the chain have a much larger area to contact with each other these are said to form trichomes

Examples → Baggiatoa

e) Palisade → If the cells are lined side by side like match stick ~~sticks~~ and at angle to one another the arrangement is said to be palisa

example - corynebacterium diptherial

ii) Hyphae → In many bacteria (eg. Streptomyces) cells are arranged to form unicellular long branched filament called Hyphae.

iii) Vibrio (sing. vibrion) ⇒ bacteria with less than one complete twist or turn and these resemble a comma (,) in appearence.
eg. vibrio cholerae

iv) Spirilla ⇒ coiled form of bacteria exhibiting twist with one or more turning giving a spiral appearence
eg. spirilla minus.

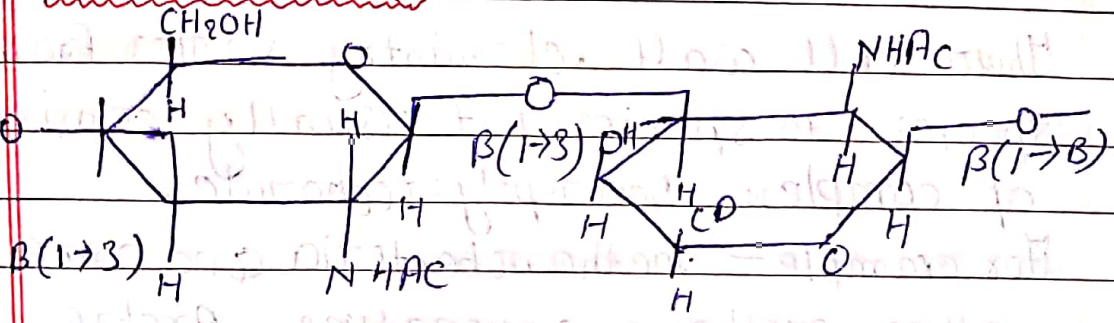
v) Other common shapes ⇒
stalked bacterium (baulobactor)
Budding bacterium (Rhodo bacterium)

vi) Pleomorphic ⇒ occurs in more than one form.
Example - Rhizobium, Azobactor, Corynebacterium, micro bacterium, mycoplasma.

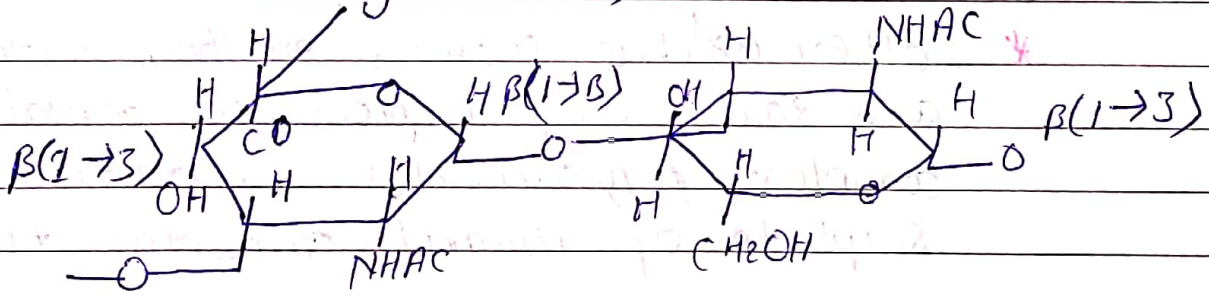
⇒ Surface appandages of Bacteria.

Surface Appendages → Flagella, Pilli, fimbriae

☆ Archaebacteria



Glu—⟨NH2⟩
|
Ala
|
Lys—Glu
| |
⟨Glu⟩ Lys
| |
⟨Ala⟩ Ala
|
Glu—⟨NH2⟩



N-acetylfolosaminuronic acid — N-acetylglucosamine

Figure:- The structure of Pseudomurein.

* Many archaebacteria have a wall with a single thick homogenous layer resembling that in gram positive bacteria.

* Their cell wall chemistry varies from species to species but usually consists of complen heteropolysaccharide.
For example - methanobacteria and some other methane generating Archae (Methanogens) have wall containing pseudomurin.

* Pseudomurien - Pseudomurin is a peptido glycan like Polymer that has L-amino acids insteads of D-amino acids in its cross links, N-acetyl talosa minuronic acid instead of N-acetyl muramic acid and B(1→B) glycosidic bond instead of B(1-4) glycosidic bond

* other archae such as methano sarcina and salt loving Haplococcus contain complex polysaccrides similar to chondrulin sulphate of Animal connective tissue.

* Difference between bacteria and Archae. -

	Property	Bacteria (Eubacteria)	Archae (Archaebacteria)
1	Nucleas	Absent	Absent
2.	unit-membrane bound organelles	Absent	Absent
3.	cell wall	Peptidoglycan containing muramic acid	verriety of types and no muramic acid
4	membrane lipid	Have ester-linked straight chain fatty acid	Have ether-linked branched disphate chain
5.	Transfer RNA (tRNA)	Thymin present. N-formulated methionine carried by initiator tRNA	No thymine in T or TψC and methionine carried by initiator tRNA
6.	Ribosome elongation factor 2	does not react with diptheria toxin	react with diptheria toxin
7.	chloramphenical	sensitive	Insensitive
8.	Anisomycin	Insensitive	sensitive
9.	DNA-dependent RNA polymerase		
a)	Number of enzymes	one	several
b)	rifampicin sensitivity	sensitive	Insensitive

10.	Polymer two type Promoter	Absent	Present
11	Metabolism		
a)	Methanogenesis	Absent	Present
b)	Chlorophyl based Photosynthesis	Present	Absent

☆ Mycoplasma

* It is a gram negative bacteria
* Mycoplasma are smallest and simplest self-reproducing gram negative bacteria.
* Smallest known bacteria are member of genus mycoplasma (about 0.3 μm in diameter)
* Mycoplasma lack cell wall and thus placed in a separate class called Mollicules (mollicus, soft, cutis, skin)
* Formerly mycoplasma were called "Pleuro Pneumonia like organism" (PPLO) because it was first isolated from cattle suffering from Pleuro pneumonia.

* Mycoplasma are pleomorphic (vary in shape) and mostly non-motile.
* Sterol are absent from the plasma membrane of all bacteria except Mycoplasma.
* Molecules similar to sterol are called **hopenoids** are present in the membrane of many bacteria
* These sterols play similar role that of plasma membrane of eukaryotic cell.
* General metabolic nature in chemoorganoheterotrophic and require cholestrol for growth
* They can be saprophytes or parasites and usually Facultative Anaerobic.
* Charactristically mycoplasma growing on solid media produce fried egg colonies with central dense region surrounded by a lighter peripheral region.
* First organism to come on Earth
* It contaminate Animal cell culture
* It can be grown on non-living media.
* It has low G+C content (heat sensitive)

* Diameter ranges from 0.3 µ to 0.8 µ.

* Size of genome is $0.7 - 1.7 \times 10^6$ bases which is half of 1/5 th to bacterial genome

* PPt or HMP and EMP (Glycolysis) Pathway occur in metabolic activity.

*

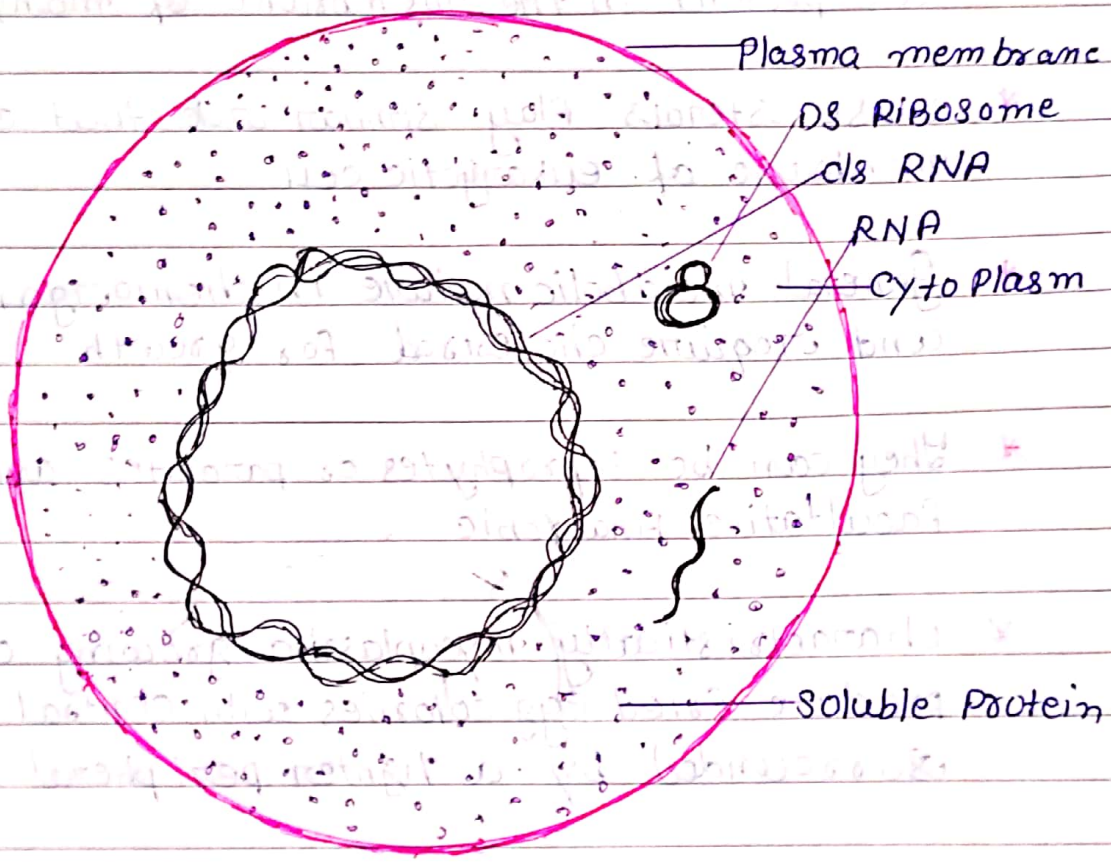


Figure :→ MYCOPLASMA

* Reckettsia

* Reckettsia is an obligate parasite

* It is a pathogens which causes thPhus Fever

* It was discovered in 1909-1910 by Howard Taylor Ricketts

* Rickettsias are Gram negative bacteria

* These are non-motile and has variable shape (Spherical, oval, rod shaped)

* Their size ranges from 0.3 Mm to 1 Mm.

* It is considered as a product of degenerative evolution (Negative evolution)

* Can't be cultured in non-living media because it cannot form ATP for Reproduction.

	Name of pathogens	Disease	Symptom
①	Rickettsiae rickettsi	Rocky mountain spotted fever	Abrupt chill, fever Rashes
②	Rickettsiae Mooseri a	Murin typhus in mice (endemic typhus)	Severe ache, fever, chills, rashes.
③	Rickettsiae akari	Rickettsial hox or vasicular rickthiosis	Similar to chicken pox
④	Coxiella burnetti	cow fever in cow to man through milk and dust	Pneumonia abrupt, chil and fever

* Rickettsias are transmitted by fleas, mites, lice and ticks.

* Rickettsias are grown in animal cell

* ## Cow fever in Cow →

Milk is Infected due to cow (cow) fever disease of Rickettsia. when human being take this milk They also suffer from this disease.

Date
Page

✱ Virus

* For the study of virus we inject it in a chicken egg which is living.

* viruses are compound of nucleoprotein (also known as necleocapsid)

NUCLEO CAPSID

- DNA
- RNA
- Protein
  - Capsomere
    - Capsid

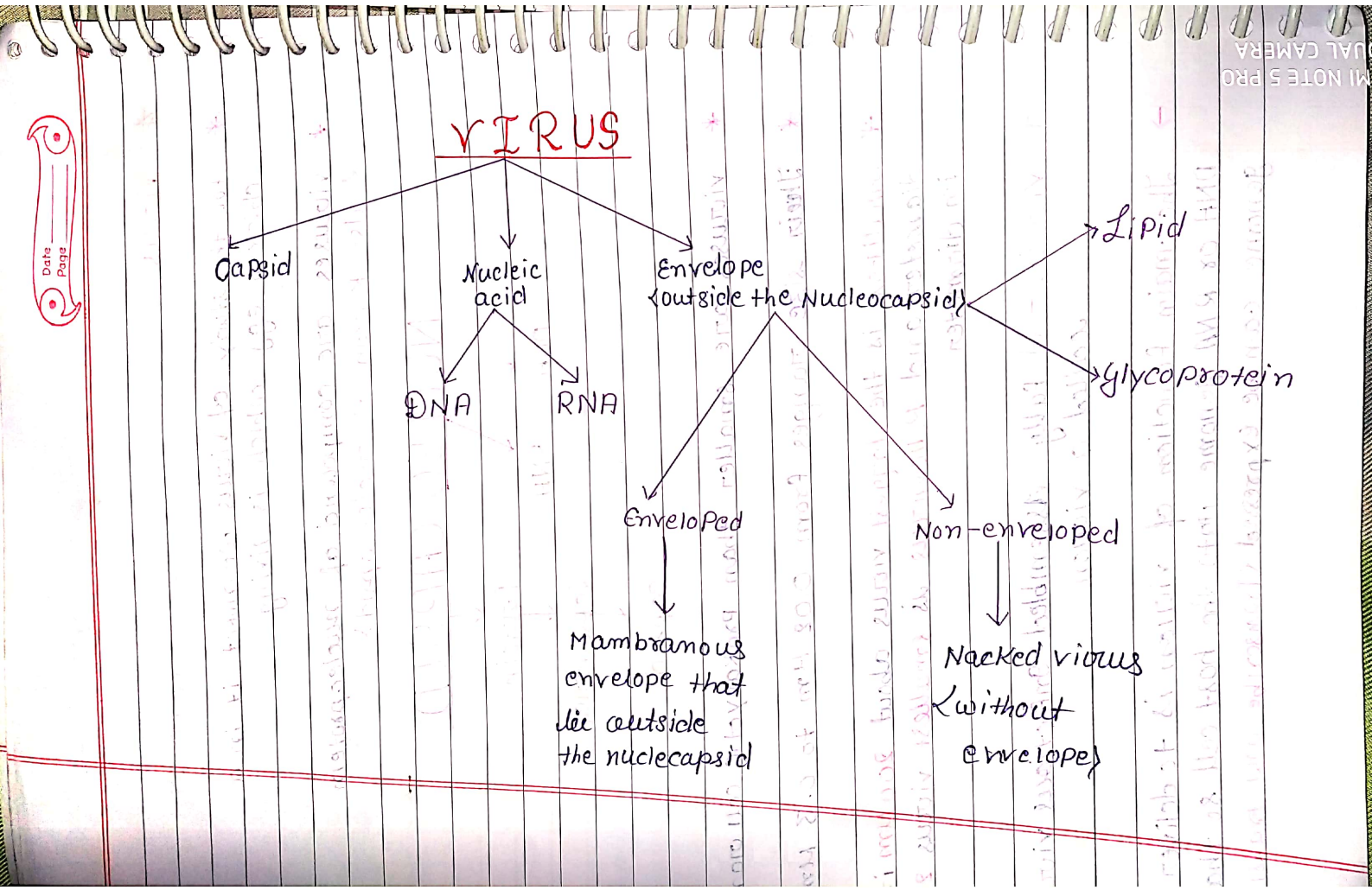
* viruses are smaller than Prokaryotic cell range

* Their size ranges from 0.02 μm to 0.3 μm.

* Small Pox is the largest virus about 200 μm in diameter and Poliovirus is smallest virus 28 μm in diameter

✱ Virions - A fully assembled Infectious virus is called a virion

→ The main function of virion is to deliver its DNA or RNA genome into the host cell so that the genome can be expressed (Transcribe and translate)



* viruses often exhibit a fringe of glycoprotein spike also called as Peplomer.

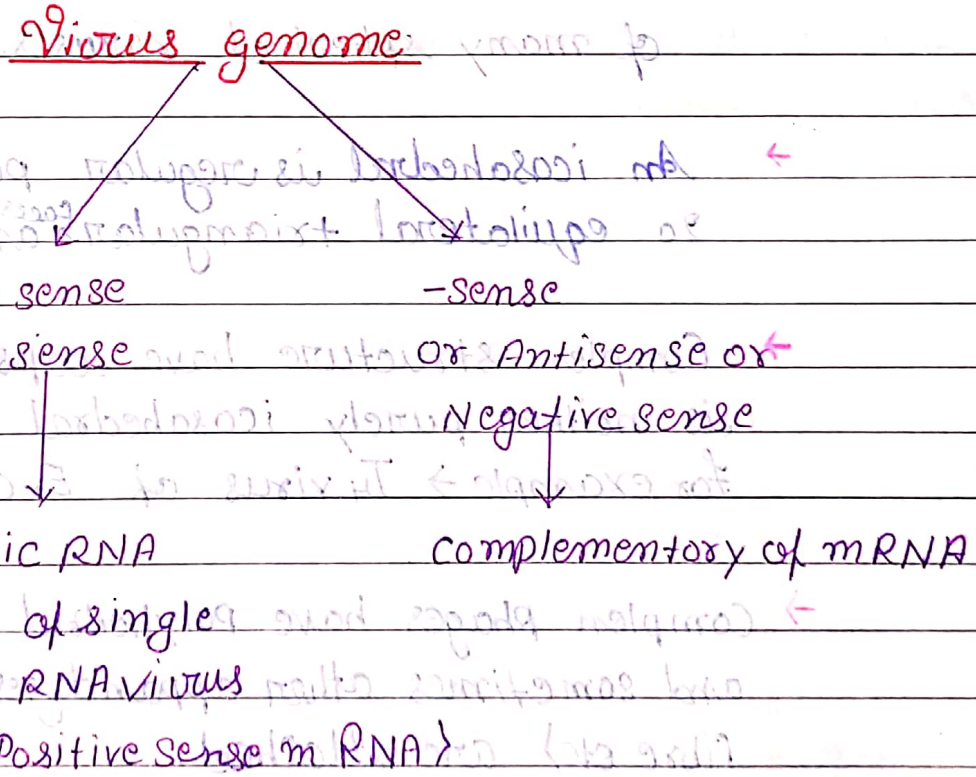
* Virus genome

→ Virus genome is devided into + sense and - Sense.

→ + sense is also known as pasitive sense or Neg: Sence while - sense is also known as negative sense or antisense

→ + Sense RNA Strand can form protein or any other product directly

→ - Sense RNA strand first prepare its complementry mRNA and then form Protein or any other product



☆ Shape / Symmetry

→ Symmetry refers to the way in which the capsomeres are arranged in virus capsid. It may be icosahedral (spherical shaped) or helical (rod-shaped).

* Helical Symmetry :→

Helical symmetry is seen in Nucleocapsid of many filamentous and pleomorphic viruses.

→ Capsomere are wrapped around a Helical filament of Nucleic acid for example, TMV

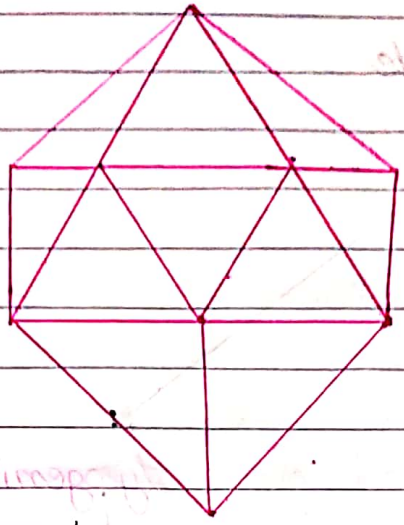
* Icosahedral Symmetry →

Characteristic nucleocapsid of many spherical viruses.

→ An icosahedral is regular polyhedron with 20 equilateral triangular faces and 12 vertices

→ Complex structure have capsid symmetry that is neither purely icosahedral nor helical for example → T4 virus of E. coli

→ Complex phages have polyhedral head to which tails and sometimes other appandages (tail plate, tail fibre etc) are attached.

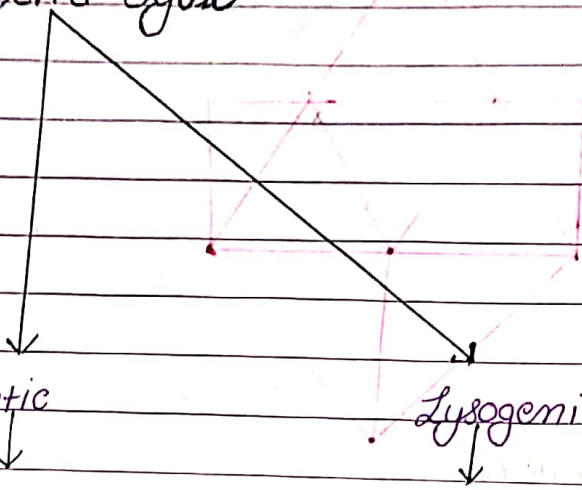


Icosahedral symmetry

☆ Types of viral nucleic acid:

Nucleic acid type	Nucleic acid structure
① DNA	
a) Single straincled	• Linear single stranded DNA • circular single stranded DNA.
b) Double straincled	• Linear double stranded DNA; Linear double stranded DNA with single chain break • circular Double DNA.
② RNA	
a) Single stranded	• Linear single stranded RNA, • Positiv Strand RNA • Linear single stranded Positive sense RNA • Linear single Stranded segmented RNA
b) Double stranded	• Linear double stranded segmented RNA.

★ Life Cycle



e.g.→ T4 Phage

e.g→ Temprate Phage

★ Burst Size →

The average number of Phage Particles produced by which each Infected cell called the burst size is characteristic for each virus and fo often ranges between 50 to several 100

★ One Step growth Curve →

one step growth curve is representation of the overall change with time in the amount of infectious virus in a single cell that the has been infected by single virus particle.

☆ Protozoa

→ Protius <Protozoa> is lobopodium

→ It is composed of ectoplasm and endoplasm

→ Pseudopodia at its forward end gets its firm consistency by Hyphae cap which is made up of ectoplasm

→ Endoplasm is devided into outer plasma gel and inner plasma sol.

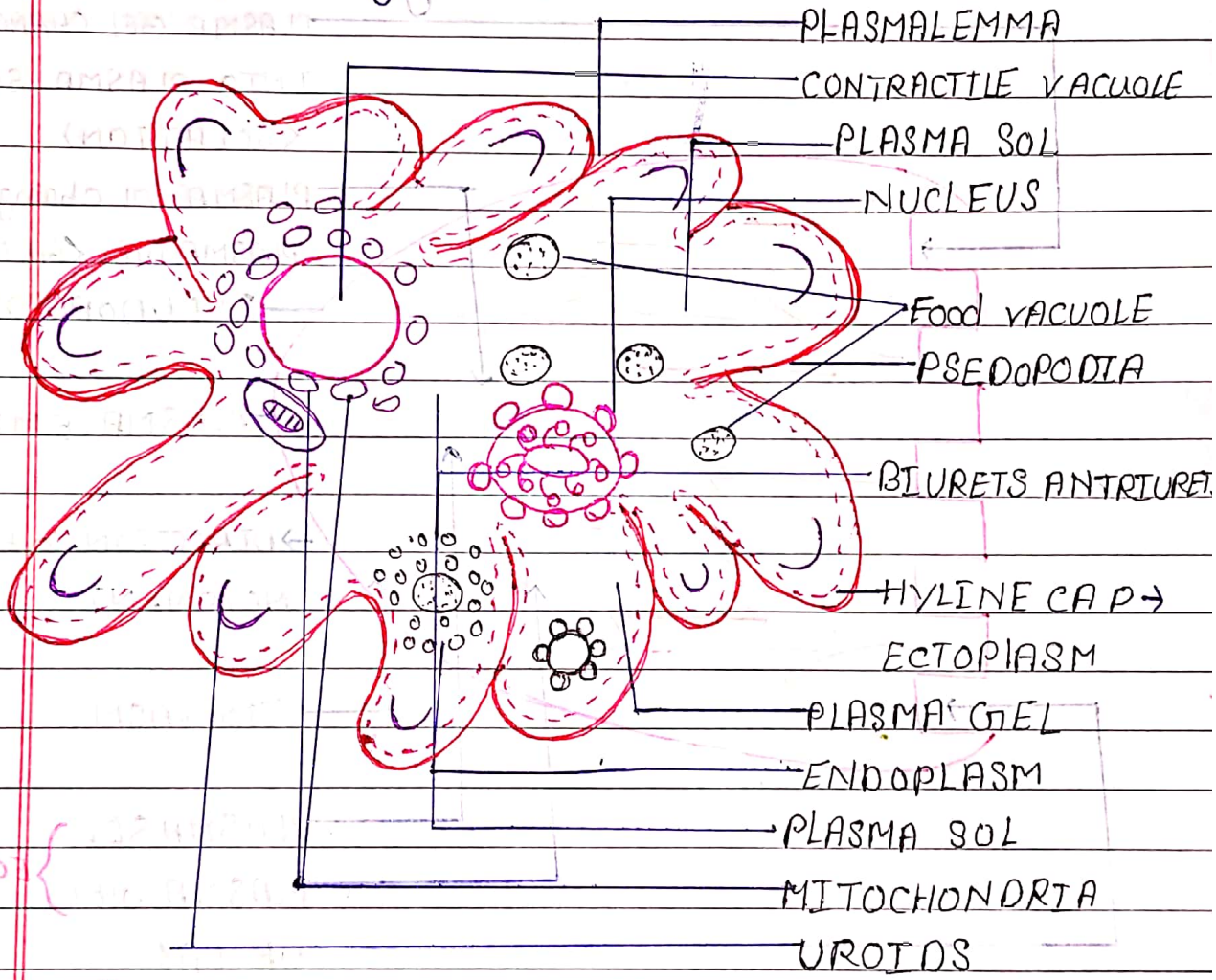


figure: structure of Ameoba

→ Sol gel theory of amoebid movement was first given by Hyman and supported by Pantenc in 1923 and masked in 1926.

→ According to this theory Amoebid locomotion due to change in viscosity of cytoplasm.

→ The conversion of plasma sol into gel and vise-versa is a physiological phenomenon.

→ Sol gel condition is due to contraction and relaxation of long chain of Protein.

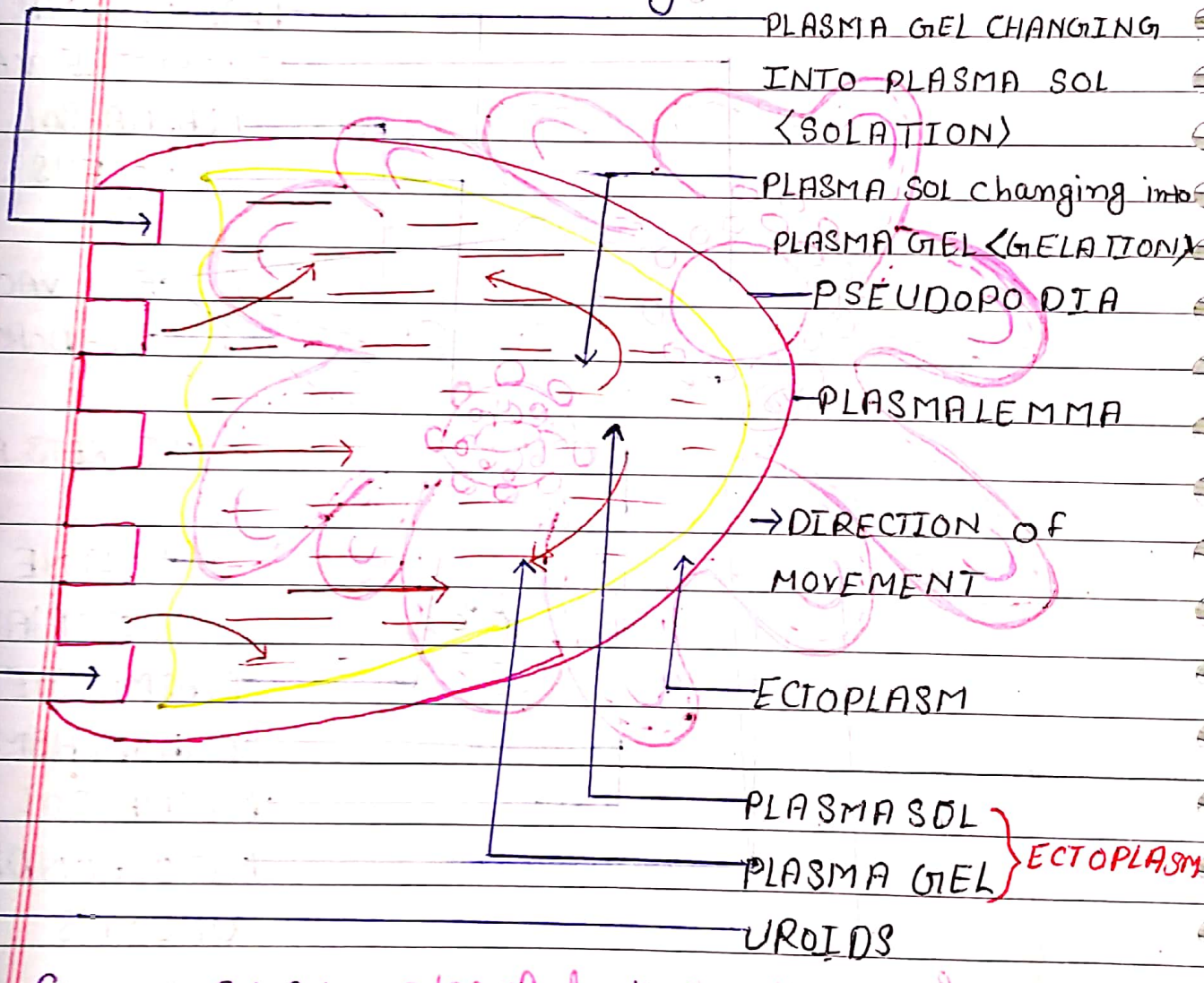


Figure :→ Sol-Gel conversion of Amoeboid movement.

☆ Contractile vacuole

1) Endoplasm of Amoeba in the posterior part contain a single clear, rounded and pulsating contractile vacuole is concerned with osmoregulation.

2) Contractile vacuole is found only in fresh water Ponds.

3) It is absent in marine and parasitic form

4) If amoeba is placed in distilled water contractile vacuole work faster.

5) If Amoeba is Placed in salt water its contractile vacuole disappear.

6) Contractile vacuole of amoeba is analogous (similar in function) to coxiniferrous tubules in frog.

7) Holozoic and Hetrotrophic mode of nutrition.

☆ Intake of food in Amoeba

* Amoeba ingest food by import circumfluences and circumvalation.

* Import → Import involves passive sinking of food into body by rupture of Plasmalemma e.g. algae.

* Circumfluances → Circumfluences is a process by which the organism ingest less active or malion less organism. e.g. bacteria.

* Circumvalation → Circumvalation is a process by which the organism engulfs active prey, e.g. ciliates or flagelates.

* Amoeba secrete digestive enzyme for hydrolysing starch, Protein and fat.

* Food vacuole of Amoeba is analogous to the alimentary canal of an animal or gastrovacular cavity of hydria.

* The content of food vacuole of Amoeba first become acidic then alkaline.

* Egestion of undigested food in Amoeba take Place through temprory rupture of the surface membrane.

* Taxis movement → Amoeba respond in enviromental condition.

→ Respond to stimuli is called taxis (movement)

→ Negative taxis - away from the stimulus.

→ Positive taxis - towards the stimulus.

☆ Bacteria

* In the 1850. The French Invastigator Casimir Davaine began calling the microscopic creatures "bacteria".

* Bacteria means 'rod-shaped'.

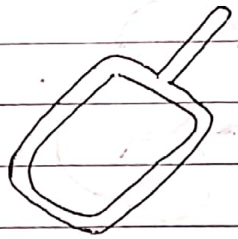
* Bacteria can be classified into different types based on their shape and size:-

1) Bacillus (rod shaped) → 0.5 μm – 2.0 μm :-

a) Singlebacillus →

b) Diplobacillus →

b) Sporeformer →



c) Streptobacillus (Strepto → chain) →

2) Coccus (sphere) [Greek word 'KOKKAS' means 'berry']

0.5 μm – 1 μm =>

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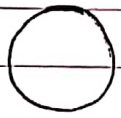
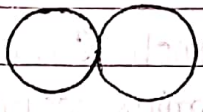
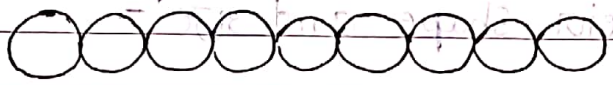
a) Single →  or monococcus

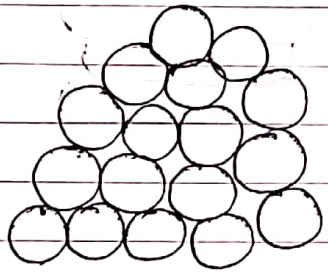
b) Diplococcus → 

c) Tetrad →  or Tetracoccus

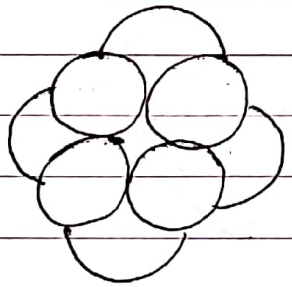
d) Streptococcus <strepto → chain> →



e) Strephylococcus <strephylo → cluster>

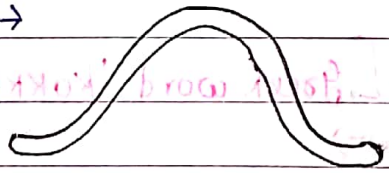


f) Sarcina <Bundle> →

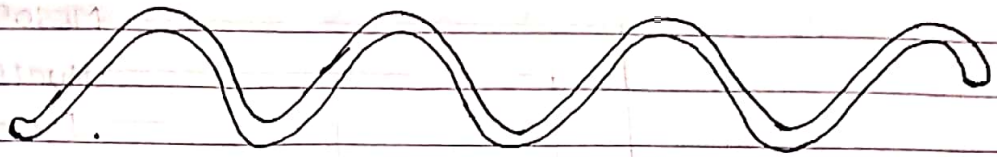


3) Spiral =>

a) Spirillium →



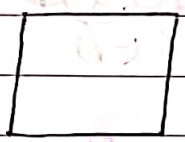
b) Spirochelate →



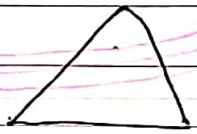
c) Vibro <comma-shaped> →

4) Others =>

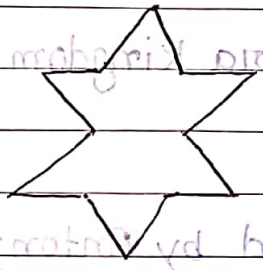
a) Square-shaped →



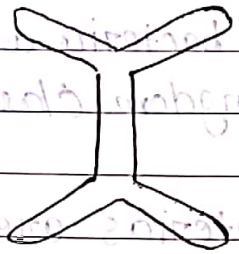
b) Triangle-shaped →



c) Star-shaped →



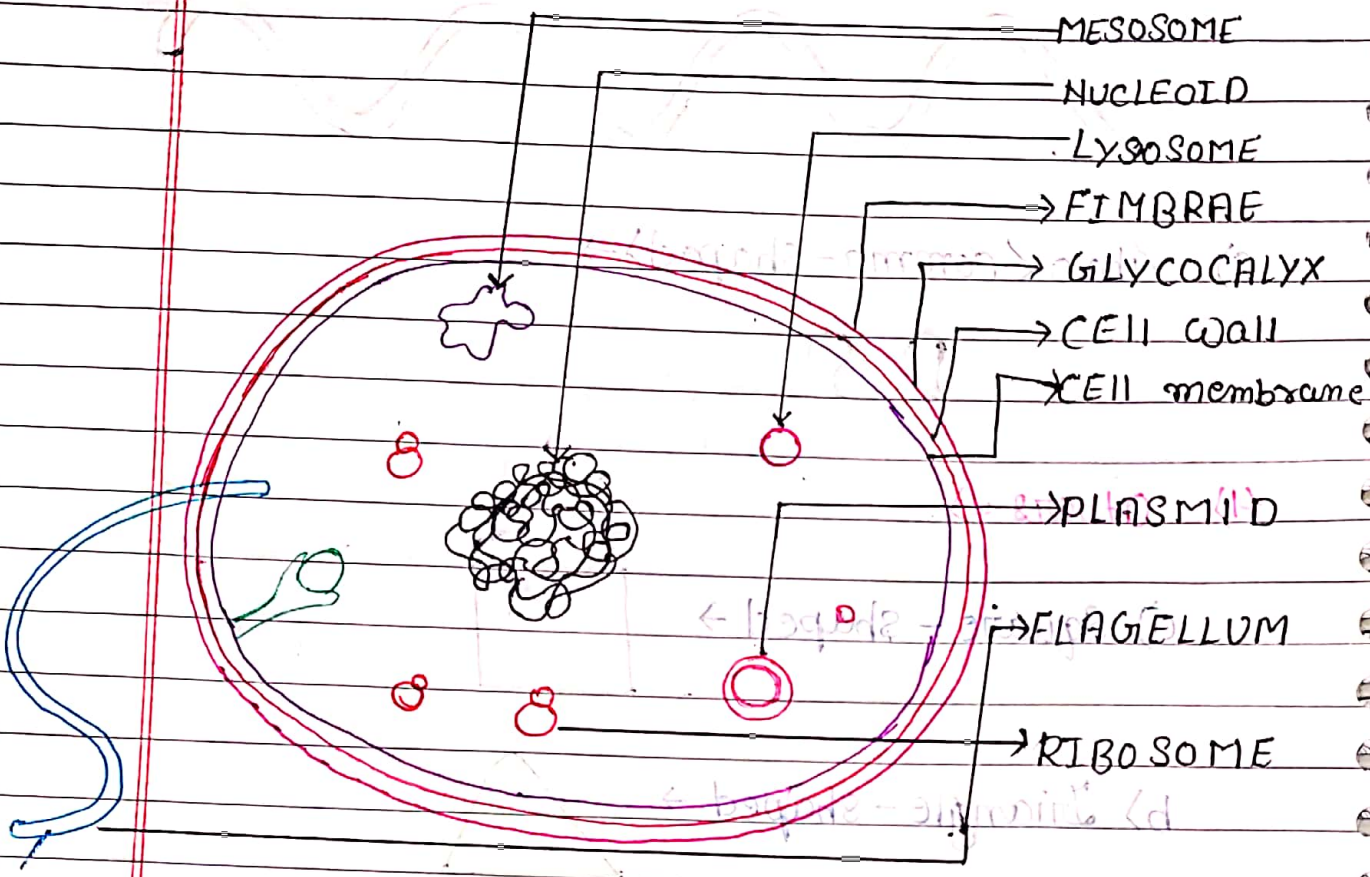
d) Mycelial bacteria :-
Mycelial-like structure



* variations in bacterial structure are readily visible when organism are magnified 1000 x under light microscope

* when electron microscope is used in magnification, 1 million times (10,00,000x) or more can be achieved.

* Structure of Bacterial cell ⇒



* Bacteria is a prokaryotic unicellular organism which is highly Primitive.

* All bacteria are Placed in Monera Kingdom of five kingdom classification.

* Bacterias are firstly discovered by Antony van Leeuwenhoek (1677). thats why he was honoured by "Father of microbiology".

* Bacteria can be found in soil, dirty water, hot water, boiling water and at every place.

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* Structure

→ Bacteria is a very small tiny organism.

→ Size varies from 0.1 Hm – 5 Hm and volume is 2.0×10^{-2} μm^3

* Shape

→ Spherical (coccus), Rod, Spiral, comma, Square etc.

* Organelles of a typical bacteria:-

1. Glycocalyx:-

→ Some bacterial cells are sorrounded by extracellular polymeric substances, which are commonly known as Capsule or Glycocalyx.

→ The capsule is gelatinous polymer made up of either polysaccharide or polypeptide.

2. Flagella:

They are composed of long rigid strand of protein subunits called Flagella.

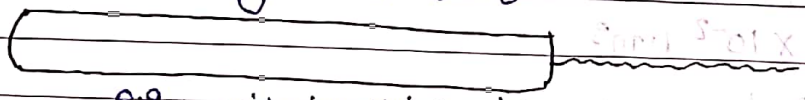
→ The Falagellum is hair-like structure emerging from the cell wall.

→ It is of 20-30 nm in diameter and 20-20μm long.

→ It provides various types of motility to the bacterial cel

→ The number and position of flagella varies from bacteria to bacteria.

→ Monotrichous → Bacteria having one flagella in any one side.



e.g. Vibrio, Thiobacillus

→ Amphitrichous → Bacteria having more than one or many flagella on both the sides.

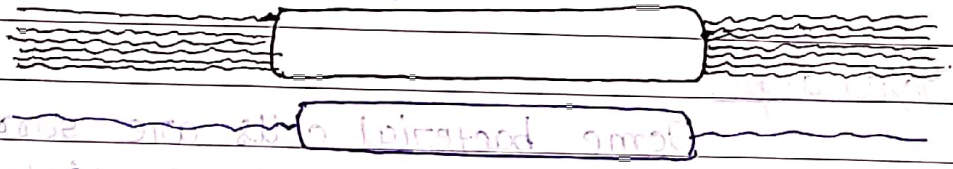
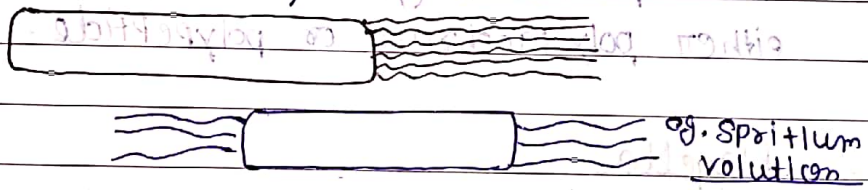


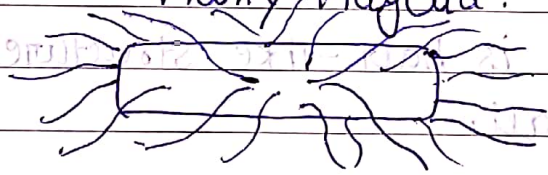
Fig: Amphitrichous. e.g:- Nitrosomonas

→ Lophotrichous → Bacteria having many flagella on any one side



e.g. Spritlum volutlon

→ Petrichous → Bacteria which is surrounded by many flagella.



e.g.- clostiridium tantani

3) Cell coall:

The outermost covering of bacteria is called cell coall which is made up of hard

rigid, non-living material that is cellulose.

4) Plasma membrane:
It is similar as eukaryotic plasma membrane which is composed of phospholipids bilayer. it contains respiratory chain enzyme, which help in respiration.

5) Mesosome:-
It is inward extension of plasma membrane, It helps in septa formation during cell devision.

6) Flagella:
It is a locomotary organ. It is hair-like structure emerging from cell wall. Its length is 10 μm - 20 μm and width is 0.2 μm.

7) Fimbrae: Fimbrae are hair-like structure found on the surface of cell wall in gram negative bacteria.

8) Ribosome: 70S type of ribosomes are found in bacterial cytoplasm. These are composed of two subunits, one is smaller subunit (30S) and other is larger subunit (50S).

→ Both are attached with the help of Mg^{2+} ions.
$1\ S = 1 \times 10^{-13}$ ~~ions~~ sec.

9) Genetic Material:
Nucleus and nuclear membrane are not found in bacteria. Therefore, genetic material is spreaded in cytoplasm called nucleoid.

→ The DNA is not associated with histone protein

10) Plasmid: plasmids are extra chromasomal DNA found in bacterial cyto plasm. DNA which contain some useful character can express according to need. such as antibiotics.

★ Flagella.

→ Present in both gram negative and gram positive bacteria.

→ Long filamentous surface appendages.

→ A flagella of gram negative bacteria such as E. coli consists of three parts:

i) Long filament - lie external to cell surface

ii) Hook - Present at the end of the filament

iii) Basal body - to which the Hook is anchored and which imparts motion to the flagellum

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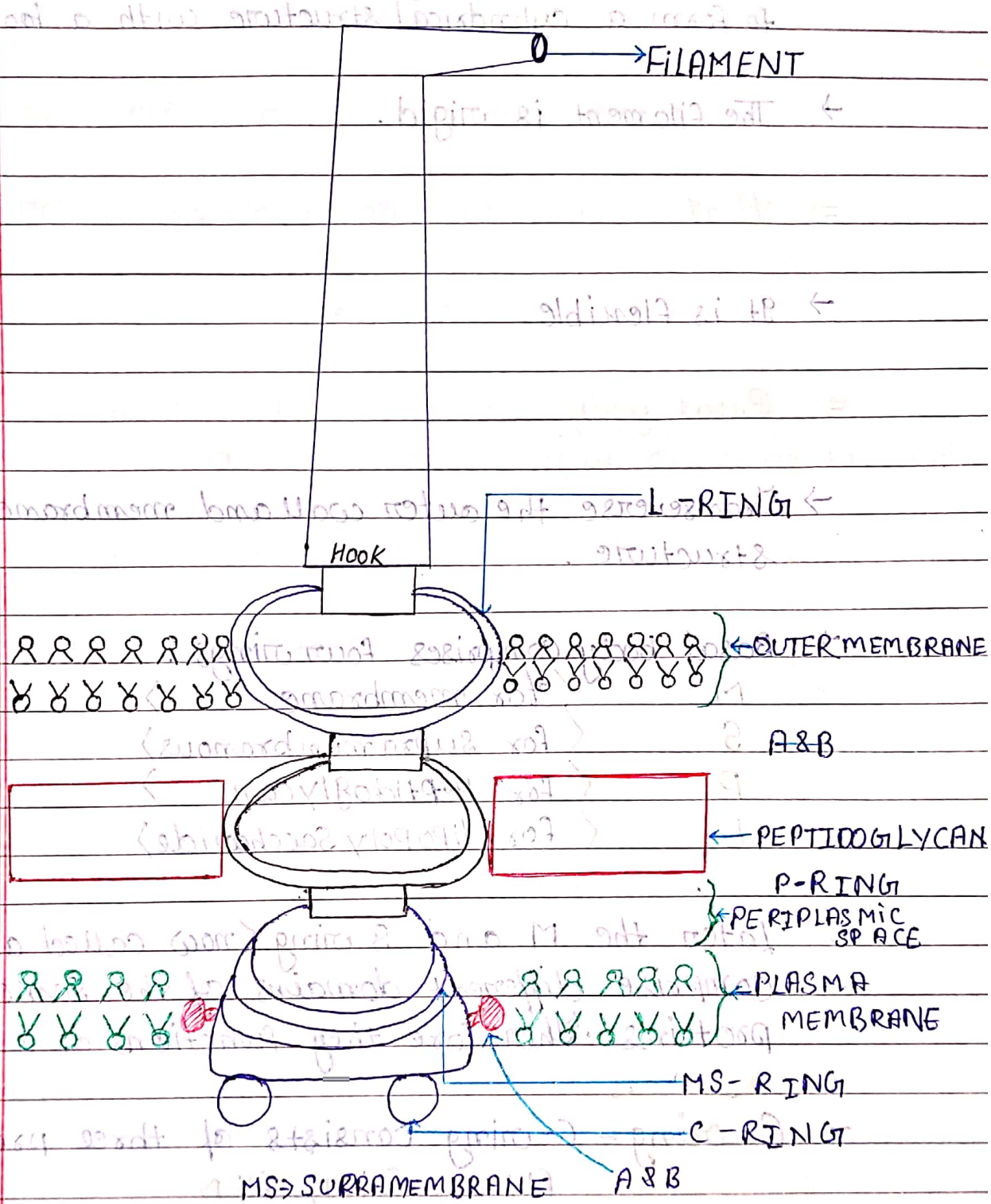
Fig.: STRUCTURE OF FLAGELLA IN GRAM NEGATIVE BACTERIA.



⇒ Filament

→ Flagellar filament is made up of flagellin protein

→ In filament the flagellin protein are assembled to form a cylindrical structure with a hollow core

→ The filament is rigid.

⇒ Hook

→ It is flexible

⇒ Basal body

→ Transeverse the outer wall and membrane structure.

→ Basal body comprises four rings:

- M ⟨for membrane⟩
- S ⟨for supramembranous⟩
- P ⟨for Peptidoglycan⟩
- L ⟨for lipopolysaccharide⟩

→ Later the M and S ring (now called as MS) comprise different domains of the same proteins. Therefore they functions as a unit

→ C-ring - C-ring consists of three proteins FliG, FliM, FliN

→ Fli G is most directly involved in the rotation of of Flagella among the C-ring protein. as it interact with the motor complex (MOT A and MOT B)

→ C-ring is also called as switch complex as it switch the direction of Flagella motor

→ BOTH MS and C-ring acts as a rotar.

→ MOT A and MOT B are arranged in a circular array (technique) around the MS and C-ring span the inner membrane.

→ MOT A and MOT B form a complex that acts as a stator and Torque generating unit.

⇒ MS + C = Rotar

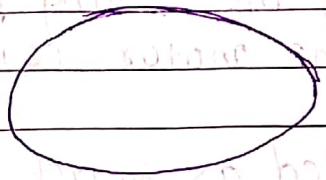
⇒ Mot A/B = Stator

⇒ Mot A/B → allows the movement of Proton along the concentration gradient.

→ regulates the direction rotation (clockwise or anti-clockwise)

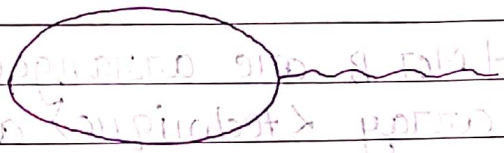
☆ Flagella Arrangement

a) Atrichous - Flagella absent



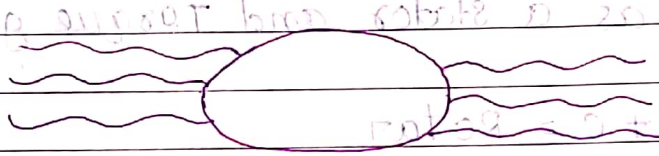
E.g - Lactobacillus

b) Monotrichous -



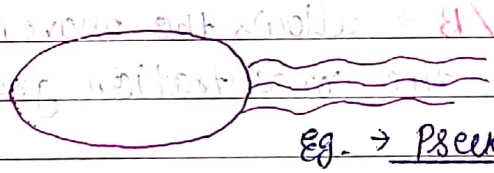
Eg → vibrio, Thiobacillus

c) Lophotrichous -



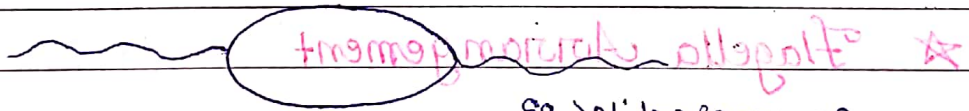
Eg → Spirillum volutan

d) Cephalotrichous -



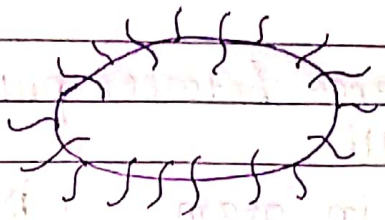
Eg. → Pseudomonas fluorescence

e) Amphitrichous -



Eg → Nitrosomonas

f) Peritrichous -



Eg → E. coli, Clostridium tentani

☆ Flagellar Motion

→ Bacterial flagellar motion is rotatory in nature.

→ The rotation of flagella can be clock wise or counter-clockwise.

→ Clockwise - forward motion cease - Tumble

→ Counter clockwise - flagella impart forward motion - Run.

☆ Prokaryotic flagella and Eukaryotic flagella.

* Difference between bacterial (Prokaryotic) flagella and eukaryotic flagella:

Bacterial flagella	Eukaryotic flagella
① Filament rigid	① Filament is flexible
② Filament is made up of flagella Protein	② Filament is made up of tubulin protein.
③ Perform rotary motion.	③ Perform whiplash motion
④ Not sorrounded by Plasma membrane	④ Sorrounded by plasma membrane

★ Difference between pilli and fimbriae.

	Pilli		Fimbrae
i)	Present in gram negative bacteria	i)	Present in both gram positive and gram negative bacteria.
ii)	Required for bacterial conjugation	ii)	used for Attachment to substratum.
iii)	one to ten per cell	iii)	1000 per cell
iv)	Long filamentous structure	iv)	short filamentous structure

★ Control of Microbial Growth

* Control of microbial growth means to inhibits or prevent the growth of microorganisms.

* The control is effected in two ways.
  i) By killing the microorganisms.
  ii) By inhibiting the growth of microorganisms

* Agents which kills cells are called cidal agents and agents which inhibit the growth of cells are reffered to as Static agents.

* Thus the term bacteriocidal reffers to killing bacteria and bacteriostatic refer to inhibiting the growth of bacterial cells.

Unit-2

"THE CONCEPT/METHOD OF STERILISATION"

★ Anti-Microbial Agents

① Disinfectant →

A chemical that kills microorganisms and eliminate it is called disinfectant.

Example → Hypochloritis, chlorine compound, copper sulphate, formaldehyde (reacts with NH_2, SH and COOH groups), Phenolic compound (denature proteins and disrupt cell membrane) and mercuric chloride (Inactivate proteins by reacting with sulphide groups)

② Antiseptic →

Antiseptics are chemical that kill or inhibit the growth of micro-organisms and are non-toxic enough to be used to living tissues.

Example → Alchohal (ethanol denature proteins and solubilise lipids), Silver nitrate (Percipitate Proteins), Iodine solution (Inactive proteins) and detergents (cell disruption or disrupt cell membrane)

③ Sterilisation →

Sterilisation refers to complete and total distruction or elimination of all viable organisms including all microbial endosp

→ Sterilisation procedure involves the use of heat, radiation or chemicals or physical removal of cells.

→ The common processes used for sterilisation are flaming and autoclaving (steam under pressure) and Tyndallysation (steam not under pressure) and filteration.

Methods of sterilization :-

(A) Dry heat (flaming, baking) -

Dry heat tends to kill microbes by oxidation of cellular component This require more energy then Protein ~~Synthesis~~ hydrolysis so higher Tem. are required for efficient sterilization by dry heat.

for example - Sterilization can normally be achieved in 15 minutes by autoclaving at 121 deg. C. whereas dry heating would generally need a Temp. of 160 deg. C to sterilize in a similar amount of time. Dry heating has one crucial differenc from auto claving. There is no need of water. so protein hydrolysis can't Take place

(B) WET HEAT (Autoclaving):-

The method of choice for sterilisation in most labs is autoclaving; using pressuried steam to heat the material to be sterilised. This is a very effective methods that kills all microbes, spores and viruses. although for some specific bugs, especially high Temp. or incubation times are required.

Autoclaving kills microbes by hydrolysis and Coagulation of cellular proteins, which is efficiently achieved by intence heat in the presence of water.

The intense heat comes from the steam. pressurised steam has a high latent heat; at 100°C it hold 7 times more heat than water at the same Tempurature. This heat is liberated with upon contact with the coller surface of the material to be sterilised, allowing rapid delivery of heat and good penetration of dense materials.

At these Tempuratures, water does a great job of hydrolysing proteins... so does bugs don't stand a chance.

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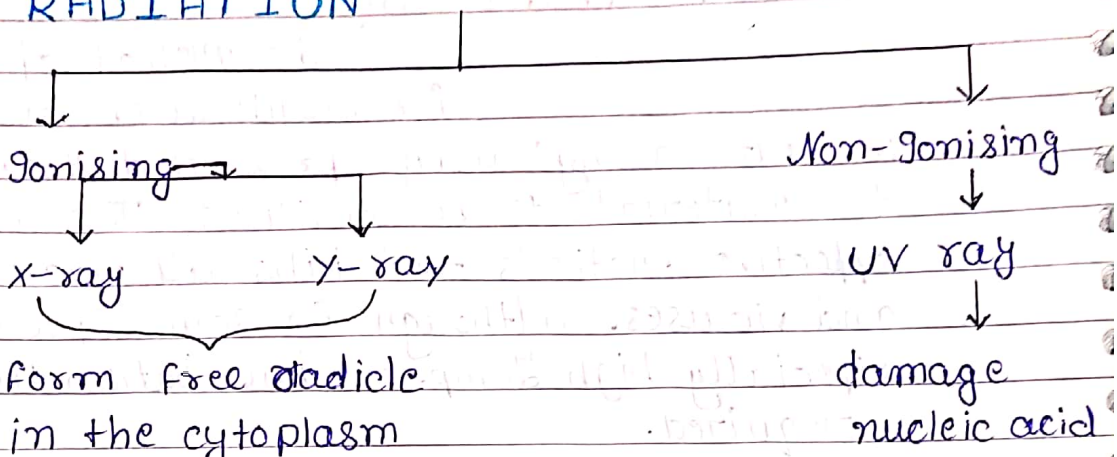
(C) RADIATION



UV, X-rays and gamma rays are all type of electromagnetic radiation that have profoundly damaging effect on DNA, so make excellent tools for sterilization.

(D) Chemical agent-

* A large number of chemicals such as alchohal, aldehyde heavy metals, halogens and dyes are commonly used as anti-microbial agents.

* Alchohal like ethyl alchohal and iso-prop alchohal cause denaturation of Proteins and are used as an antiseptic.

* The heavy metals mercury and silver

are used as an anti microbial agent.

* Mercury (Hg) is used as mercuric chloride and as a component of merchurochrome.

* Silver (Hg) is commonly used as silver nitrate.

* Two important halogens, chlorine and Iodine acts as oxidising agents that react with amino acid in proteins and change the nature of Proteins.

Similarly gases like ethylene oxide, formaldehyde and β-propeolactone are used to acieve sterilization.

(F) Filteration -

Filteration is a physical removal of all cells in a liquid or gas. It is specially important for sterilisation of solution which would be denatured by heat (e.g. antibiotics, amino acids, vitamins etc)

- In this process solutions or gases are passed through a filter of sufficient core diameter (generally 0.22 μm) to remove the smallest known bacterial cells.

- Filteration is carried out commonly by using

membrane filters.

- Membrane filters are made up of cellulose esters (cellulose acetate, cellulose nitrate, collodion etc)
- A range of pore ~~size~~ sizes are available.
- Bacterial filters have a pore size of less than 0.75 μm.

(G) Tyndallization →

Tyndallization is a process used to culture media that might be spoiled by exposer at higher Temp.

- vegetative bacterias are killed
- Any spores that survive that will germinate in nutrient medium over night. producing vegetative forms that are killed by second and third steam.

UNIT-3

MICROBIAL GROWTH

* Bacterial growth is an orderly increase in the quantity of cellular constituent that is cell mass and number
* It depends on the ability of the cell to form new protoplast from nutrients available in the environment.
* The increase in bacterial cell number occuss by cell devision which is known as binary fission.
* Binary fission begins when the DNA of the cell is replicated.
* Each circular strand of the DNA then attaches to the plasma membrane.
* The cell elongates causing the two replicated DNA to seprate
* The plasma membrane then invaginates (group inward) and splits the cell into two daughter cells through a process called cytokinesis.
* Bacteria segragate DNA as it replicate and lack a eukaryote-like mitotic apparatus for segratting chromosomes
* A few bacterial speciles reproduce by budding.

→ They form a small bud that enlarges until its size approaches that of its parent cell and then it seprate.

⇒

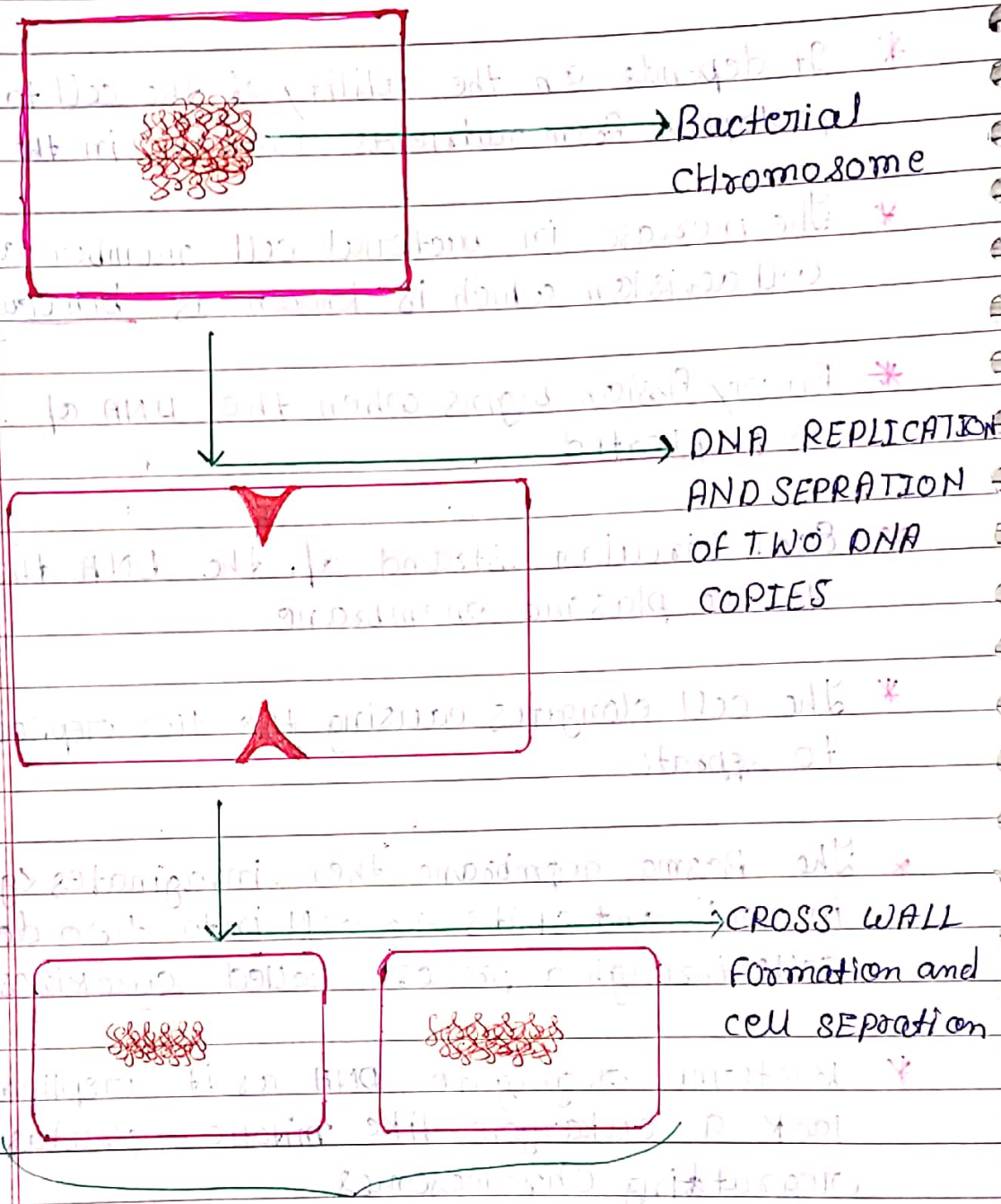


Fig: Devision of bacteria by BINARY Fission.

* Fits Z

→ Fits Z Protein Plays an important role in cell devision.

→ fits z is homologous to tubulin.

→ Tubulin is the building-block of microtubles in eukaryotes.

→ The cellular of concentration of Fits Z regulate the frequency of division.

→ At the line of septum imagination the fits Z protein is transferred the cytoplasm to devision site where it assemble into a ring that remains associated with the leading edge of the imaginating septum until the sepration in completed.

* There are four stages in bacterial growth.

i) Lag Phase → There is no Increase in cell number.

→ It is a period of adaptation of cells to new environment.

→ There is no change in number but increase in mass.

ii) Log Phase or Exponential Phase →

→ Exponential phase of growth is a pattern of balance to growth

→ All the cells are dividing regularly by binary fission and by geometric progression 1, 2, 4, 8, etc or $2^0, 2^1, 2^3 \ldots 2^n$ where n = number of generation

→ The cells devide at a constant rate depending upon the composition of the growth medium and the condition of incubation.

The rate of exponential growth of a bacterial culture is expressed as generation time also called doubling time of the bacterial population.

The generation time is the time interval required for the cells (or population) to devide.

(iii) Stationary Phase →

→ Exponential growth can't be continued forever in a batch culture (eg. a closed system)

→ In this phase the cell growth has leveled off and become constant.

→ The number of cells multiplying is equal to the number of cells dying.

→ Population growth is limited by one of the three factors:

a) Exhaustion of available nutrients.

b) Accumulation of inhibitory metabolites or end products.

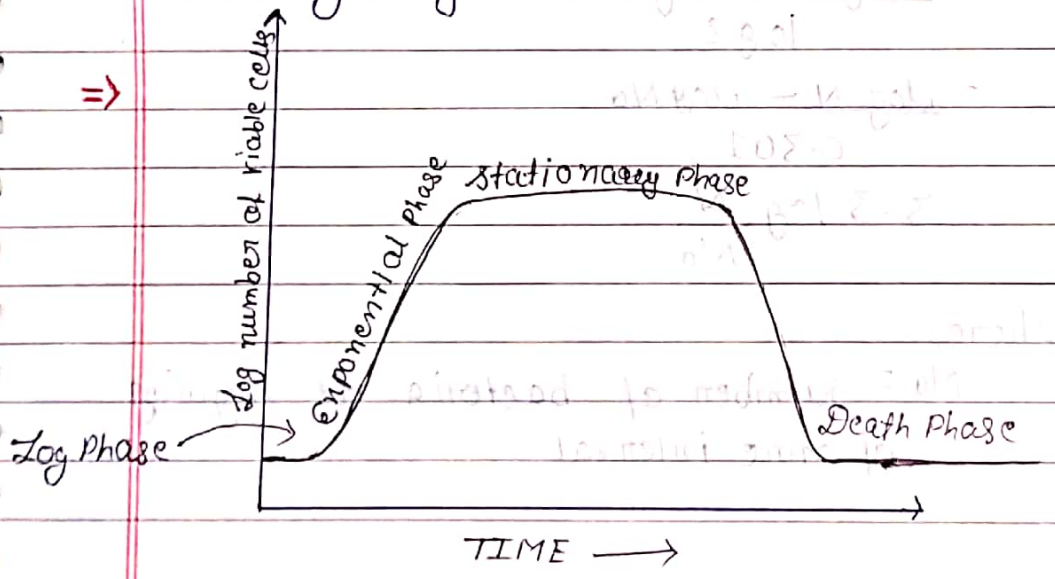
c) Exhaustion of space, in this case called a lack of biological space.

iv) Death Phase →

→ Decline in cell population.

→ The number of viable cells decreases geometrically (exponentially) which is reverse of the growth during log Phase.

=>



★ Calculation of generation time.

* **Generation Time →**

* If we start with one cell, when it devides there are two cells in the first generation, four cells in the second generation, eight cells in the third generation and so on.

* The interval for the formation of two cells from one is called generation and the time required for this to occur is called generation time.

* $$\text{generation time} = \frac{t \text{ (time in minutes or hour)}}{n \text{ (number of generation)}}$$

* $N = N_0 \times 2^n$

Applying logrithum

$$\log N = \log N_0 + n \log 2$$

$$n = \frac{\log N - \log N_0}{\log 2}$$

$$= \frac{\log N - \log N_0}{0.301}$$

$$= 3.3 \log \frac{N}{N_0}$$

where,

N_0 = number of bacteria at begining of time interval

N = Number of bacteria at the end of the time interval

n = number of generation

★ Calculation of bacterial cell density

* Cell density is the number of cells per unit volume.
* The number of bacterial cell can be determined directly as well as Indirectly.
* Direct measurement can be ~~determined~~ carried out be measuring colony number of by counting the number of cells in known volume with a microscope.
* where as indirect method involves the measurement of optical absorbance through spectrophotometer.

* Measurement of colony Number.

$$= \frac{\text{Number of colonies formed}}{\langle \text{millilitre plated} \rangle \times \langle \text{dilution before plating} \rangle}$$

$$= \frac{\text{Number of viable cells}}{\text{millilitre of individual culture}}$$

A. Effect of bacterial response to environmental factor

	Term	definition	example
1.	Halophiles	Require high level	Halobacterium

		of salt concentration	
2.	Acidophiles	growth optimum between pH 0 and 5.5.	Sulpholabus
3.	Alkalophiles	Growth optimum between pH 8.0 and 11.5.	Bacillus alkalophiles
4.	psychrophiles	Grow well at 0°C and has an optimum growth temperature of 15°C or lower.	Chlymodomonas nivilis
5.	psychrotroph	Can grow at 0 to 7°C, has an optimum growth between 20 and 30°C.	pseudomonas flusescence
6.	Thermophiles	Can grow at 55°C or higher optimum growth often between 55 to 65°C.	Thermus aquaticus
7.	Hyperthermo-philes	Has an optimum temperature often between 80 to 110°C.	pyrococcus and pyrodictium
8.	obligate aerobes	Does not require oxygen for growth	Bacillus Subtilis
9.	obligate anaerobes	Can perform only anaerobic respiration.	clostridium botulinum
10.	Facultative aerobes	Anaerobic form	Chlorobium

but can live in the presence of oxygen.

11.	Facultative anaerobes	Aerobic form but can live in anaerobic condition also.	pseudomonas.
12.	Anaerotolerant anaerobes	bacteria continue to perform anaerobic respiration even in the presence of oxygen.	Lactic acid bacteria and streptococcus pyogens.
13.	Anaerotolerant aerobes	Aerobic bacteria continue to perform aerobic respiration even in the absence of free oxygen by using oxygen of oxidised salts.	Denetrifying bacteria
14.	Microaerophile	Require oxygen level below upto 10% for optimum growth.	Treponema pallidum

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☆ Historical gene transfer and genetic Recombination

* gene transfer refers to the movement of genetic Information between organism.

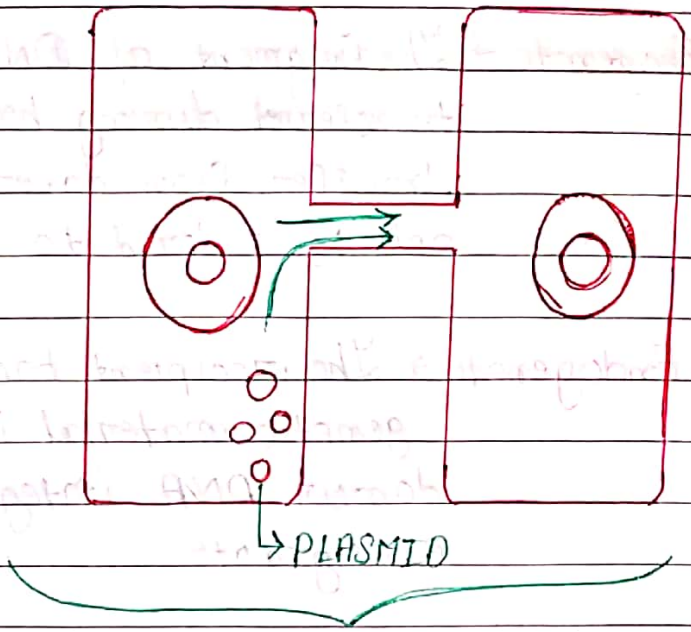
* gene transfer can be horizontal and vertical

* Transfer of genes from Parients to offspring is termed as vertical gene transfer whereas transfer of gene between two Indicpendent organism is called horizantal or lateral gene transfer

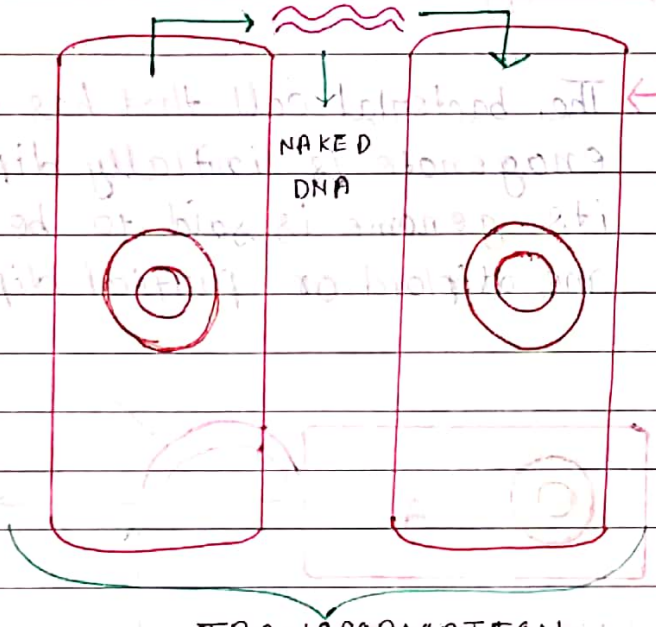
* Example of vertical gene transfer :

i) Sexual reproduction in eukaryotes.
ii) Binary fission in prokaryotes.

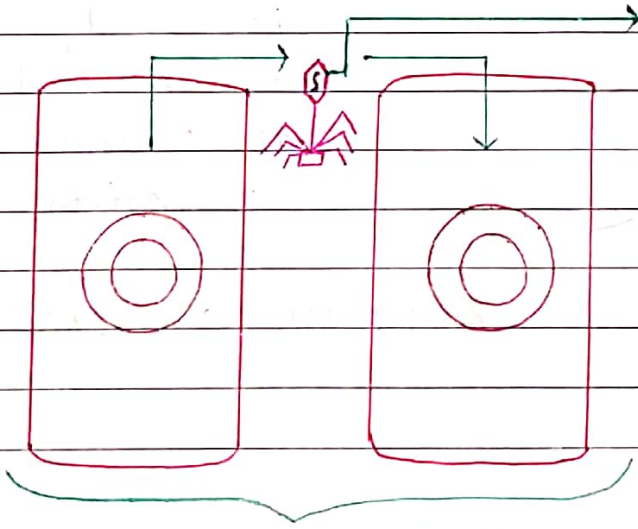
* Example of horizontal gene transfer :
i) Transformation
ii) Transduction
iii) Conjugation



CONJUGATION



TRANSFORMATION



TRANSDUCTION

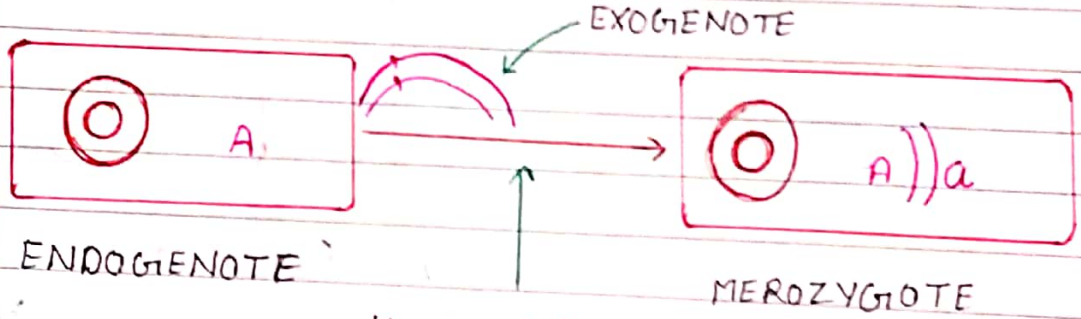
☆ Exogenote → The fragment of DNA that has been transferd during horizontal gene transfer from doner cell to recipent cell is refered to as an exogenote

☆ Endogenote → The recipient bacterial cells own genetic material into which the donor DNA integrate is called endogenote

V.V.I ☆ Merozygote → (Patriot diploid or merodiploid).

→ The bacterial cell that has recieved an exogenote is initially diploid for part of its genome is said to be merozygote or merodiploid or partial diploid

⇒



Horizontal gene transfer
transformation,
transduction and
conjugation

FORMATION OF MEROZYGOTE

→ In merozygote gene (allele) of exogenote may or may not substitute of alleles of endogenote.

→ The process of replacement of one allele by different alleles from the same genes by preserving the structure of gene is termed as genetic recombinatio

→ Exogenote are normally degraded rapidally so that there is essentially A race between the process of degradation of the exogenote and recombination

Transformation

* Griffith Experiment

→ In 1928, Griffith performed a Transformation experiments with two different strains of the bacterium diplococcus pneumoniae (now called as Streptococcus pneumoniae) virulent strain which vertebrates (such as mice, human) and avirulent strains which not cause pneumonia.

→ The difference in virulence is related to the polysaccharide capsule of the bacterium.

→ Virulent strains have this capsule while avirulent strains do not

→ The non-capsuled bacteria are readily engulfed and destroyed by phagocytic cell.

→ Virulent bacteria, which posses the polysaccharide capsules are not only easily engulfed. Hence or they are able to multiply and cause Pneumonia

→ Encapsulated bacteria form smooth colonies (S) when grocon on cm agar culture plate whereas non-incapsulated strains produce rough colonies (R)

→ Each strain of diplococcus may be of different serotype (different strains).

→ Serotype are identified by immunological technique.

→ Griffith perform experiments with two different strains IIR and IIIS

Serotype	Morphology	Capsule	virulence
IIR	Rough	Non-capsulated (absent)	Avirulent
IIIS	Smooth	Capsulated (present)	virulent

→ Griffith inlected the different strains of bacteria into mice

I. Live IIR (Non-virulent, non-capsulated) } When injected into mice → mice survived

II. Live IIIS strain (virulent, capsulated) } Inject into mice → mice dies.

III. Heat-killed IIIS strain (Non-virulent) } when injected into mice → mice survived.

IV. Heat-killed IIIS Strain and live IIR Strain } when injected into mice → Mice dies.

→ Griffith concluded that the heat-killed IIIS bacteria were responsible for converting live avirulent IIR cells into virulent IIIS ones and called the phenomonic tranformation.

He called the genetic information which could be passed from the dead IIIS cells to IIR cell 'the transforming principle'.

* Avery-Mac Leod-Mc Carty experiment in 1944.

→ In 1944, oswald Avery, colin Mac leod and Maclyn Mc garty revisited griffith's experiments and concluded that transforming material was pure DNA not protein or RNA.

→

I. IIR ⟶ No transformation.

II. Type IIR cells + IIIS DNA extract → Transformation

III. Type IIR cells + type IIIS + DNase

↓

No transformation

IV. Type IIR + type IIIS + RNase

↓

Transformation

V. Type IIR + Type IIIS + Poteinase

↓

Transformation

☆ Competence

* Transformation may be natural or artificial.
* Natural transformation is a very rare event and has been observed in both gram positive (Streptococcus, Pneumonial, Bacillus subtilis) as well as in gram negative (Heamophilocs influenza)
* The ability of recipient bacteria to take up free DNA and become transformed is known as competence.
* Some species of bacteria are naturally competent.
* Transformation can occur at high frequency since most cells in a population can take up environmental DNA at any time.
* Competent bacteria that can take up DNA encode proteins called competence factor.
* These proteins facilitate the binding of DNA fragments to cell surface and uptake of DNA into the cytoplasm.
* Transformation in Homophiles influenza the first gram negative bacteria in which natural competence was found.

* In Heamophilous influenza DNA uptake is associated with the formation of a small membranous structure called transformosome which protrudes outside the cell.

* The transforming DNA is taken into these vesicles where it is internalised into the cell.

* DNA uptake in Haemophilous influenzae appears to require specific sequence termed uptake sequences, for example -
AAGTGCGGTCA in
Heamophilous influenzae.

* The Heamophilous influenza take up DNA of their own species by recognising uptake sequences whereas other species such as Streptococcus Pneumonial and Bacillus subtilis do not discriminate between their own and foreign DNA.

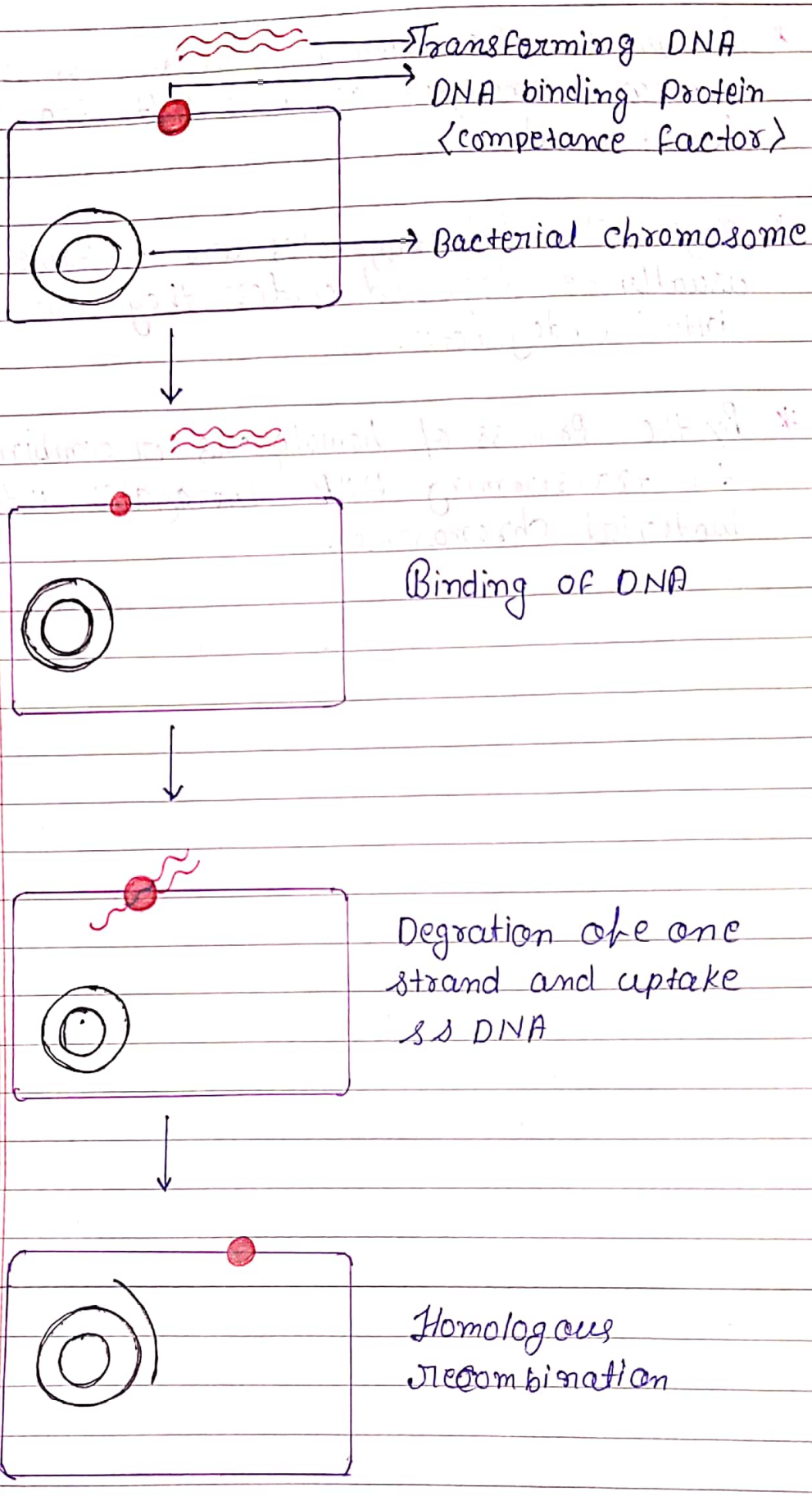
☆ Process of transformation

* Once the free DNA comes in the contact with the competent bacteria, linear, double stranded DNA enter into the cell but one strain is degraded while the other is integrated into the chromosome by homologous recombination.

* Recipient cells undergo this process and acquire a new phenotype as a result are said to be transformed.

* Single stranded enogenotes are unstable and will usually be degarded unless they are intergated into endogenote.

* By the Process of homologous recombination, the transforming DNA intergates into the bacterial chromosome.



✱ Mapping by transformation

* Transformation is used for gene mapping.
* Mapping by transformation experiments is based on the principle that two genes tranform together if they are near enough to be carried on the same DNA fragment.
* ~~if two genes~~ Lets consider two genes A and B present on the bacterial chromosome
* If two genes A and B are widely seprated in the chromosome that they are always carried on the two difficult DNA fragments.
* If the two genes are so near one another that they are often present on a single DNA fragment.
* The frequence of simultaneous transformation (co-transformation) is same as the frequency of single gene transformation. Thus the frequency of co-transformation is inversly proportional to the distance between the two genes.

✱ Transduction

* Bacteriofages functions as a vector to transfer DNA from donar bacteria to recipeint bacteria.
* Transfer of bacterial gene by phage was discovered

by Laderberg and Zinder in 1951 in Salmonella species.

* In Salmonella typhimurium, there are two types of transduction.

i) Generalised transduction.
ii) Specialised transduction.

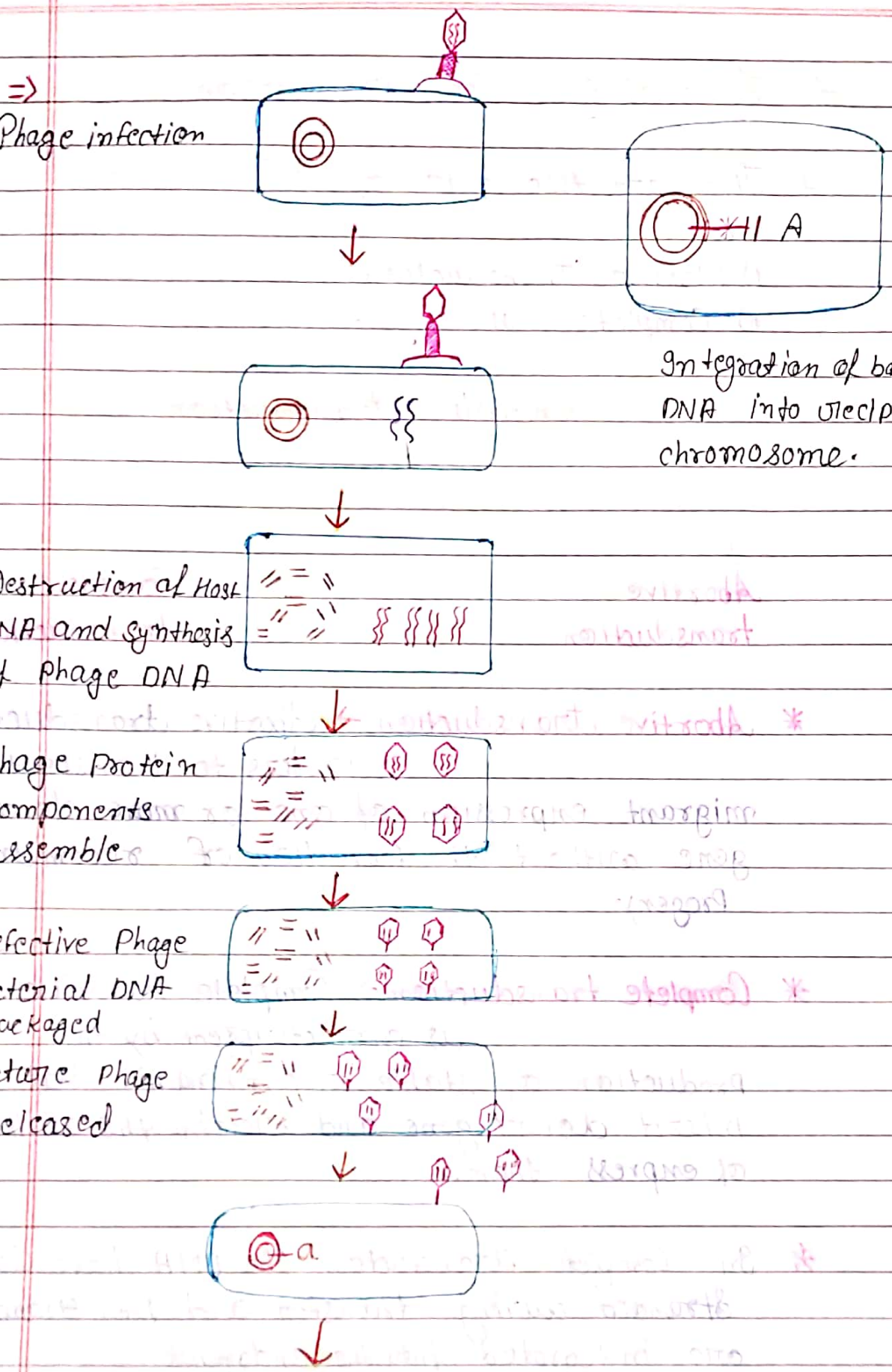
☆ generalised transduction.

* Transducing phages produced during lytic growth and aberrant / abnormal and contain a random fragmentation of bacterial genome instead of phage DNA.

* Each individual transducing phage carries a different set of closely linked gene representing a small sigment of bacterial genome.

* When a generalised transducing phage infects, a recipient cells expression of the transfer donar gene occur.
Example - P1 (E.coli) and P22 (Salmonella)

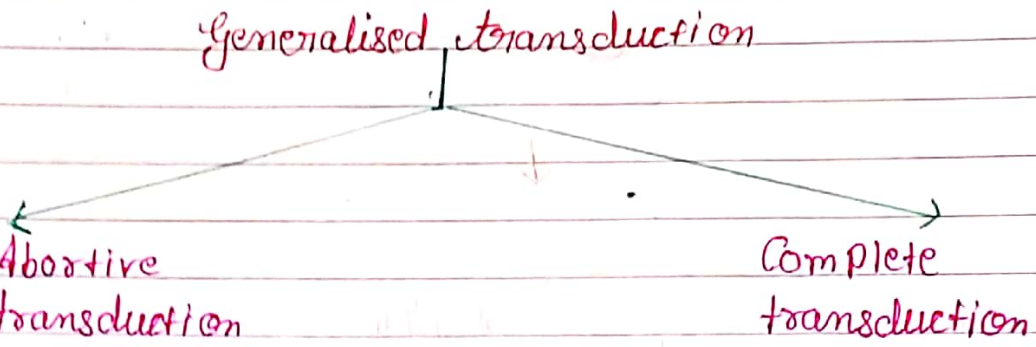
Fig: Generalized Transduction.



☆ Types of Generalised transduction

* There are two type of G.T.

i) Abortive Transduction.
ii) Complete "



* Abortive transduction → Abortive transduction refers to transient / migrant expression of one or more donor gene without the formation of recombinant Progeny.

* Complete transduction → Complete transduction is characterized by the Production of stable recombinants that inherit donor gene and retain the ability of express them.

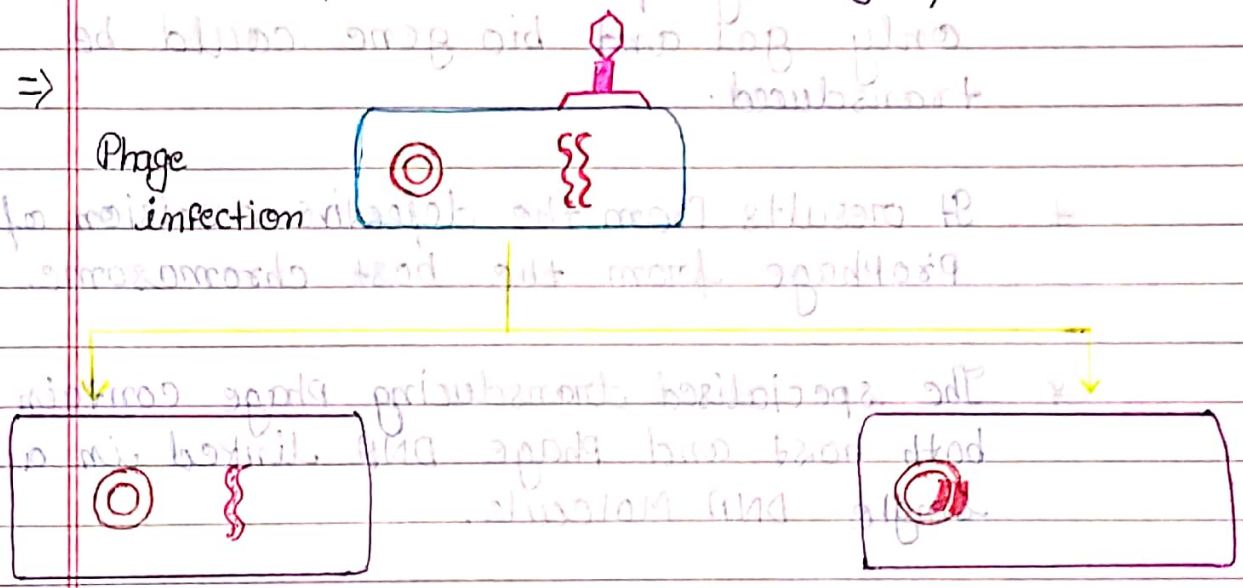
* In Complete tranduction the DNA have double Stranded during transfer and both strands are integrated into the endogenate

* In abortive transduction the donar DNA fragment does not integrate with endogenote and also not replicate

* progeny of original transductant bacteria contains the donar DNA fragment in abortive transduction

* The frequency of abortive transduction is typically 1 to 2 orders of magnitude greater than frequency of complete tranduction indicating that most cells infected by generallised tranduction phages do not produce recombinant progeny.

⇒



* Specialised transduction

* It is mediated by specific tempurature phage.

* Specialised transducing phages are formed only when lysogenic donar bacteria enter the lytic cycle and release Phage Progeny.

★ Character of Specialised (restricted) transduction

* The only bacterial gene that can be transduced are those very near the site at which prophages are integrated.

* For example, site at which lambda (λ) phage integrates into the host chromosome is between the gene for galactose fermentation (gal) and biotene Synthesis (bio). so if the prophage disintegrates abnormally from the host chromosome only gal and bio gene could be transduced.

* It results from the defective excision of prophage from the host chromosome.

* The specialised transducing phage contain both host and phage DNA linked in a single DNA Molecule.

* They are rare recombinant which lack part of the normal Phage genome and contain part of the bacterial chromosome located adjacent to the prophage attachment site

* Formation of lambda gal (λ gal) and lambda bio (λ bio) transducing Particles causes loss of some λ gene

* λ gal Particles lack the tail genes and sometimes head genes.

* Both of which are located at the right - end of the prophage.

* The λ bio particles lack genes from the left-end of ProPhage < Int for Integrase and xis for excisenase>

* The number of missing Phage genes depends on the position of the cut that generated the particles.

* The head and tail genes are essential so λ gal transducing particle are unable to perform lytic Cycle and thus plaque.

* They are defective and this is denoted by λd gal.

* The gene missing in λ bio tranding Particles are not essential for lytic growth.

* So λ bio Phages usually plaque forming and are called λP bio, where P stands for Plaque forming.

* If λd gal transducing Particle lack Phage genes required to grow typically the transducing particle is produced by aberrant excision

From the normal Prophage.

* The Prophage contains all the essential genes and hence the necessary gene products (head and tail proteins) is still present in the chromosome.

⇒ Integration of Phages

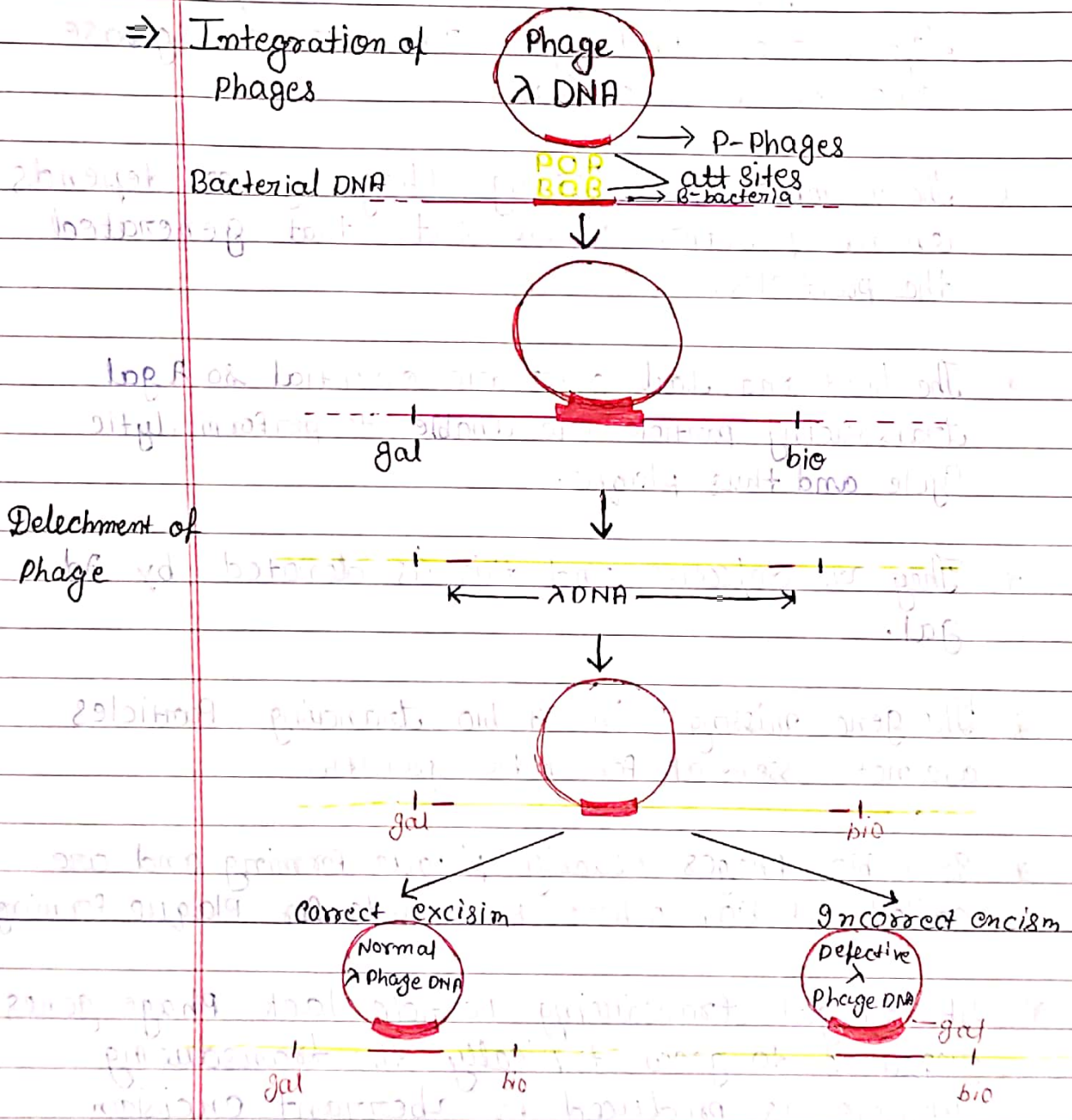
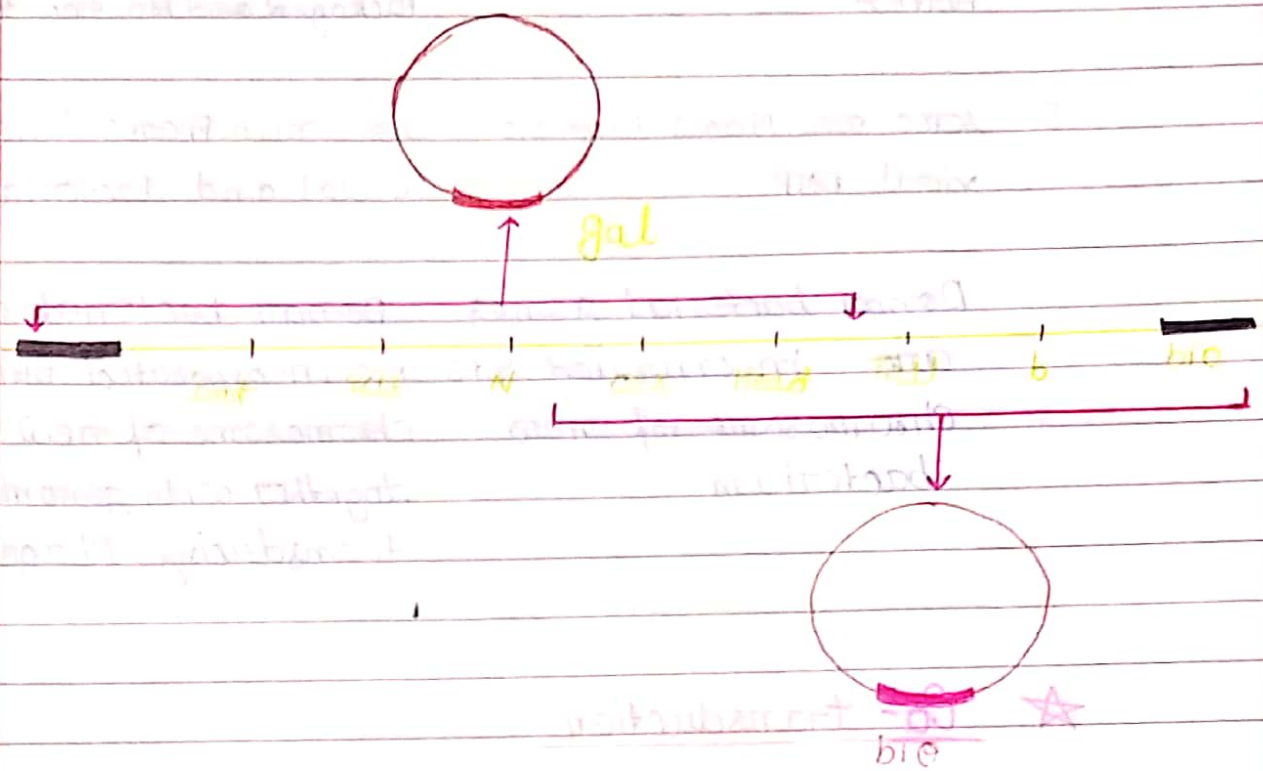


Fig: The Production of Defective Lambad (λ) Phage

* If a bacterial cell is double infected with a wild type (normal) lambda (λ) phage and a λd gal the wild type phage can supply the function missing in the defective phage and the progeny will contain about equal type number both types.

⇒



☆ Difference between generalised transduction and Specialised transduction

generalised transduction	Specialised transduction
1) occurs during lytic cycle of virulent and temp. phage	occurs during the lysogenic cycle of temperature phage.
2) Phages penetrates the bacterial cell and enter lytic cycle	Phages penetrates the bacterial cell and enter lysogenic cycle.

3)	Viral DNA begins to replicate immediatly in the bacterial cytoplasm	Viral DNA integrates into the DNA of the bacterium and replicate them later.
4)	Any bacterial genes are randomly Packaged into new Phage Particle	Bacterial genes adjacent to Previously Incorporated virus are Packaged and into new Phage Particle
5)	some new Phages have no viral DNA	Some new Phages have both viral and bacterial DNA.
6)	Donar bacterial genes are incorporated into Chromosome of new bacterium	Donar bacterial genes are incorporated into the Chromosome of new bacterium together with genome of transducing Phage.

☆ Co-transduction

* If two genes are close enough along the chromosome, a bacteriophage may be Package a single piece of chromosome that carries both genes and transfer that piece of to another bacterium.

* Co-transduction depends on how close they lie.

* If two genes are far apart along the bacterial

chromosome they can never be co-transduced because the bacteriophage can't physically package a DNA fragment that is too large.

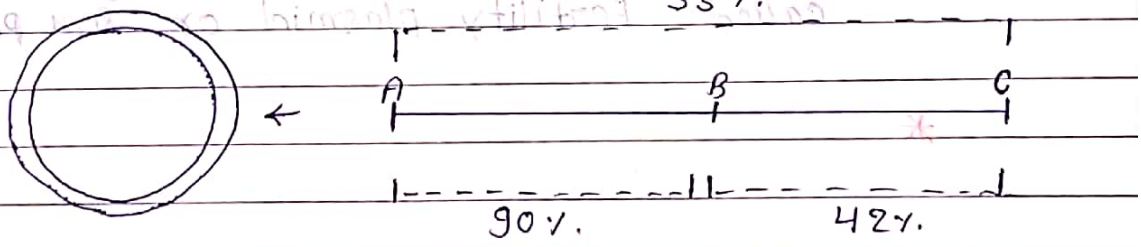
* The co-transduction can alloco be used to map genes present in the bacterial chromosome.

* Mapping of genes by the process of co-transduction is based on the fact that closer the gene to each outer greater the probability of their co-tranduction.

* The frequency of co-transduction is inversly propotional to the distance between two genes.

* For example-

→ Consider three genes A, B and C for which the frequency of co-transduction determined by two factors crosss are ~~upto~~ A to B - 90%, B to C - 42%, A to C - 33%.



$$C = \left(1 - \frac{D}{L}\right)^3$$

where,

C= Co-transduction frequency.

D= distance in minutes between two genes

L= size of transducing fragment in

✱ Conjugation

* In 1946 Joshua lederberge and Edward Tautum showed that bacteria undergo conjugation.
* In conjugation direct contact between the donar and recipent bacteria leads to the establishment of cytoplasmic bridge between them and transfer of a part or all the donar genome to the recipient takes place
* Donar ability is determined by the presence of self-transmissible / conjugative plasmid called fertility plasmid or sex plasmid.
*
*

* Self-transmissible plasmid generally exist in both gram positive and gram negative bacteria.

* A recipient cell that has recieved DNA as a result of conjugation is called trans conjugation

* Transfer of plasmids is generally intra-specific. However many plasmid transfer system that enable them to transfer DNA between unrelated species, this is known as Promiscuous plasmid.

* F^+ to F^- Conjugation

→ F Plasmid (also called F factor) of Escherichia coli is a Prototype for fertility plasmid in gram negative bacteria.

→ The conjugative functions of plasmids are specified by a cluster of atleast 25 transfer (tra) genes which determine the expression of sex Pilli, synthesis and transfer of DNA during mating and another functions.

→ The tra gene can be devided into two groups.

i) Those whose products are involved in the mating pair formation (MPF)

ii) Those whose products are involved in processing the plasmid DNA for transfer (Dtr).

→ The mpf component includes a sex pillis that extrucks from the cell and holds mating cell together.

→ The MPF system also includes the channel in membrane through which DNA and Protein Pass

→ Each F+ bacterium has 1 to 3 Sex pilli that binds to a specific outer membrane protein on recipient bacteria to initiate mating.

→ An inter-cellular cytoplasmic bridge is formed and one strand of F plasmid DNA is transferred from donar to recipient begining at a unique origin and processing in the 5' → 3' direction.

→ The site on the plasmid DNA at which transfer initiates is called the origin of transfer <oriT>

→ Relaxase protein makes a single strand cut at oriT site in the plasmid.

→ The transeferred strand is converted to circular double clouble stranded F Plasmid DNA in the recipient bacterium.

→ Both of the conjugants bacteria are F^+ and the F plasmid can therefore spread by infection among genetically compatible populations of bacteria.

* Hfr to F^- conjugation.

→ Donar strand with integrated F plasmid can transfer chromasomal gene to recipient with high efficiency, they are called Hfr (High frequency recombination) strains.

→ In case of mating between Hfr and F^- strain, Part of F plasmid is transferred at last after the entire bacterial chromosome has been transferred.

→ Because only a part of F plasmid is transfer at the start, the F^- does not become F^+ unless the whole chromosome is transferred.

→ Complete transfer takes place in about 100 minutes in E. coli and the conjugation usually break before the process is finished. Thus a complete F plasmid is not transferred and the recipient remains F^-.

* F' to F^- Conjugation

→ Segments of bacterial chromosome can become incorporated into hybrid F plasmid, this is called as

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F' Plasmid.

→ F factor is an episome and can integrate into bacterial chromosome.

→ The integration is reversible it can leave the bacterial chromosome.

→ When the F factor leaves the Hfr chromosome it ocassionally picks up some bacterial genes to become F' plasmid

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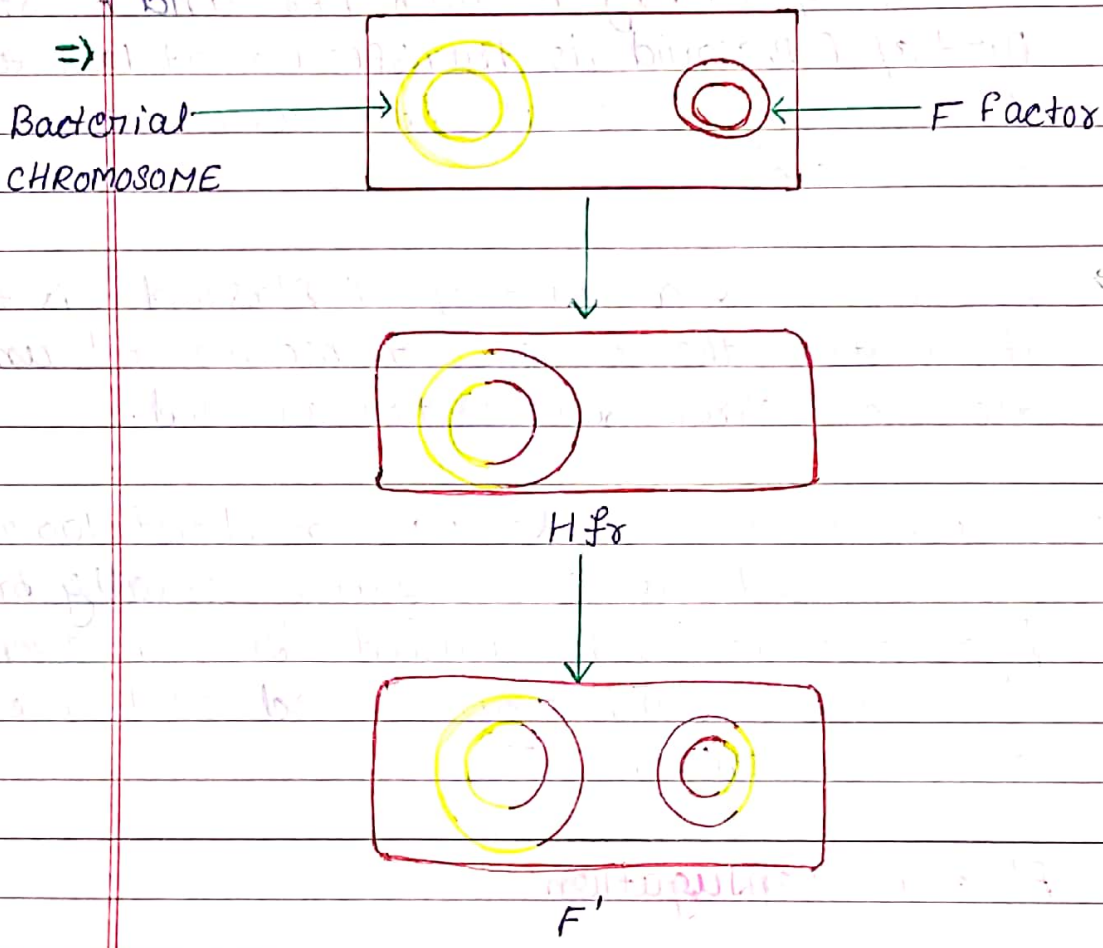


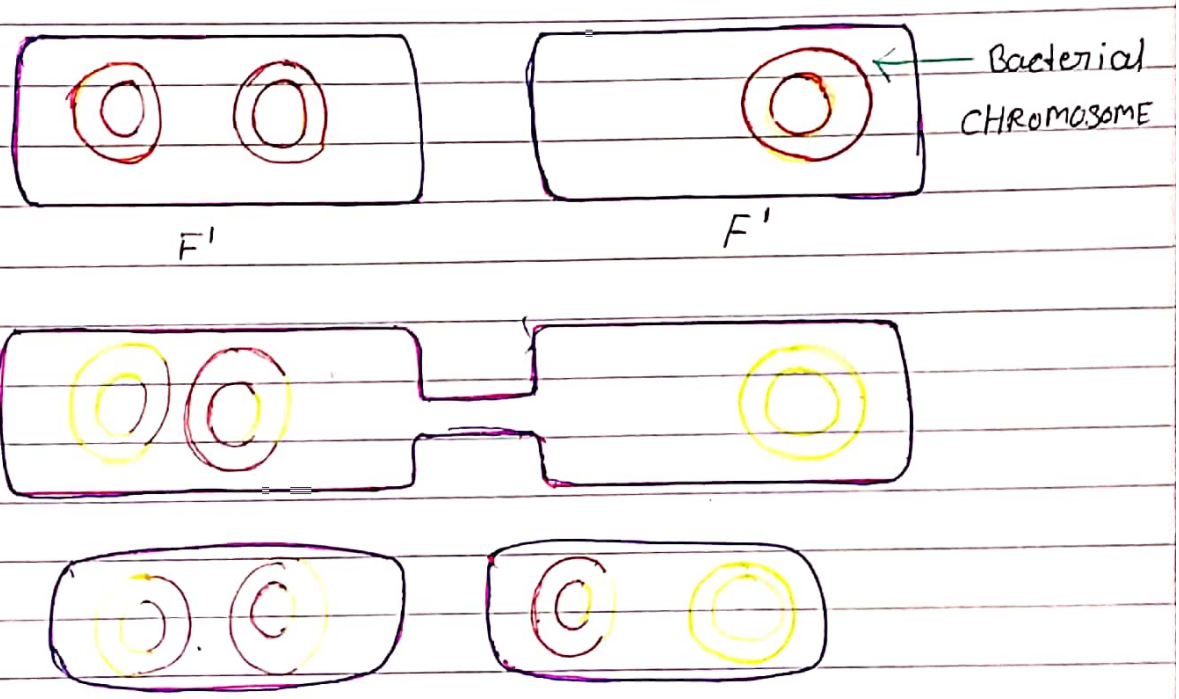
→ F' to F⁻ conjugation is identified/e to F⁺ to F⁻ conjugation

→ Bacterial genes on F' Plasmid are transferred with it and need not to be incorporated into the recipient chromosome to be expressed.

→ The recipient become F' and is partially diploid merozygote.

→ Since it has two sets of genes carried by the plasmid, such transfer of bacterial genes is often called as Senduction.

⇒



UNIT - IV

☆. Control of Microbial Growth

- Control of microbial growth means to inhibit or prevent the growth of microorganisms.
- The control is affected in two ways.
- i) By killing the microorganisms.
  ii) By inhibiting the growth of Mos.
- Agents which kills cells are called cidal agents and agents which inhibit the growth of cells are referred to as static agent.
- Thus the term bactericidal refers to killing bacteria and bacteriostatic refers to killing bacteria the inhibiting the growth of bacterial cells.

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☆. Anti - Microbial Agents

1. Disinfectant →

A chemical that kills Mos and eliminate it - is called disinfectant.

example - hyphochlorilis, chloxine compound, copper sulphate, formaldehyde < reacts with NH_2, SH and COOH groups >, phenolic compound < denature proteins and disrupt cell membrane > and mercuric chloxide < inactivate proteins by reacting with sulphide groups. >

2. Antiseptic →

Antiseptics are chemical agents that kill or inhibit the growth of microorganisms and are bacteria and extend the milks usable life.

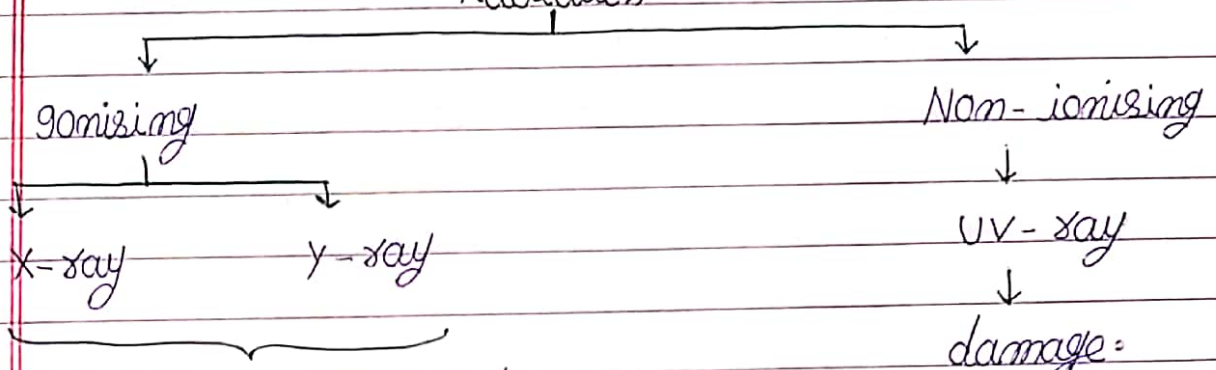
☆. Microbial control method

1.> physical agent -

a> Heat → Dry → oxidation of large molecule.

Moist → Coagulate and Denaturation.

b) Radiation



from free radicle in the cytoplasm and the free radicle damage microbial protein and DNA.

c) Filtration -

Filtration is a physical removal of all cells in a liquid or gas. It is specially important for sterilization of solution which would be denatured by heat (eg:- antibiotics, amino acids, vitamins etc).

- In this process solutions or gases are passed through a filter of sufficient core diameter (generally 0.22μ) to remove the smallest known bacterial cells.

- Filtration is carried but commonly by using membrane filters.

- Membrane filters are made up of cellulose esters (cellulose acetate, cellulose nitrate collodian etc).
- A range of pore sizes are avaible.
- Bacterial filters have a pore size of less than 0.75 μ.
- React with amino acids in proteins and change the nature of protein.
- Similarly gases like ethylene oxide, formaldehyde and β-propeolactone are used to achieve sterilization.

2.) chemical agents -

- A large number of chemical such as alcohol, aldehyde, heavy metals, halogens and dyes are commonly used as anti-microbial agents.
- Alcohol like ethyl alcohol and iso-propyl alcohol cause denaturation of proteins and are used as an antiseptic.
- The heavy metals mercury and silver are used as an anti-microbial agents.

- Mercury (Hg) is used as mercuric chloride and as a compound of mercurochrome.
- Silver (Ag) is commonly used as silver nitrate.
- Two important halogens, chlorine and iodine acts as oxidising agent that

⇒ Tyndallisation →

- Tyndallisation is a process used to culture media that might be spoiled by exposes at higher temperature
- Vegetative bacterias are killed.
- Any spores that survive that will germinate in nutrient medium over night, producing vegetative forms are killed by second and third steam.

☆. pasteurization

- pasteurization is a brief heat treatment used to reduce the number of spoilage organisms and to kill disease causing microbes.
- Milk is usually pasteurized by heating at 63°C for 30 minutes (batch method) or at 71°C for 15 minutes (flash

* Milk is usually pasteurized by heating at 63°C for 30 minutes (batch method) or at 71°C for 15 minutes (flash method) to kill bacteria and extend the milk's usable life

* **Staining →**

Staining is a biochemichal tehniaue. Dyes are used to stain the spicemen

→ Dyes are used to stain the specimen.

→ Dyes are organic compound consisting of two functional chemical groups, one chemical group gives dye its characteristics colour while the another group contains an invisible chemical structure which helps to solubilise the dyes and facilitates its binding to different structures

Dye → Cationic / Basic
Dye → Anionic / Acidic

Cationic
[e.g - crystal voilet, malachite green, methylen blue, safranin]
↓
will react with group that have negative charge

Anionic
[e.g → eosin, acid fuchsin]
↓
will react with group that have positive charge

→ Bacterial staining protocaal can be devided into three basic types:

i) Simple staining
ii) Differential staining
iii) Specialised staining

I) Simple staining – Stain reacts uniformly with all cell type and only distinguish the organism from their surroundings.

II) Differential staining – Stains do not stain all types of cell with the stain colour it discriminates different cell types depending upon the chemical and physical composition of the cell.
The differential stain is more frequently used for Bacteria either gram stain and acid fast – stain.

III) Specialised staining – Detect specific structures of cell such as flagella and endospores

* Gram staining (Gram's methods) →

The method is named after the name of inventor the Danish Scientist Hans Cristian Gram who developed the technique in 1884

→ The bacteria was first stained with basic dye crystal voilet (Primary stain) which imparts Purpledecolour to all cells

→ Bacteria is then treated with gram's iodine solution. This allows the stain to be retain better be forming an insoluble crystal voilet - iodine complex.

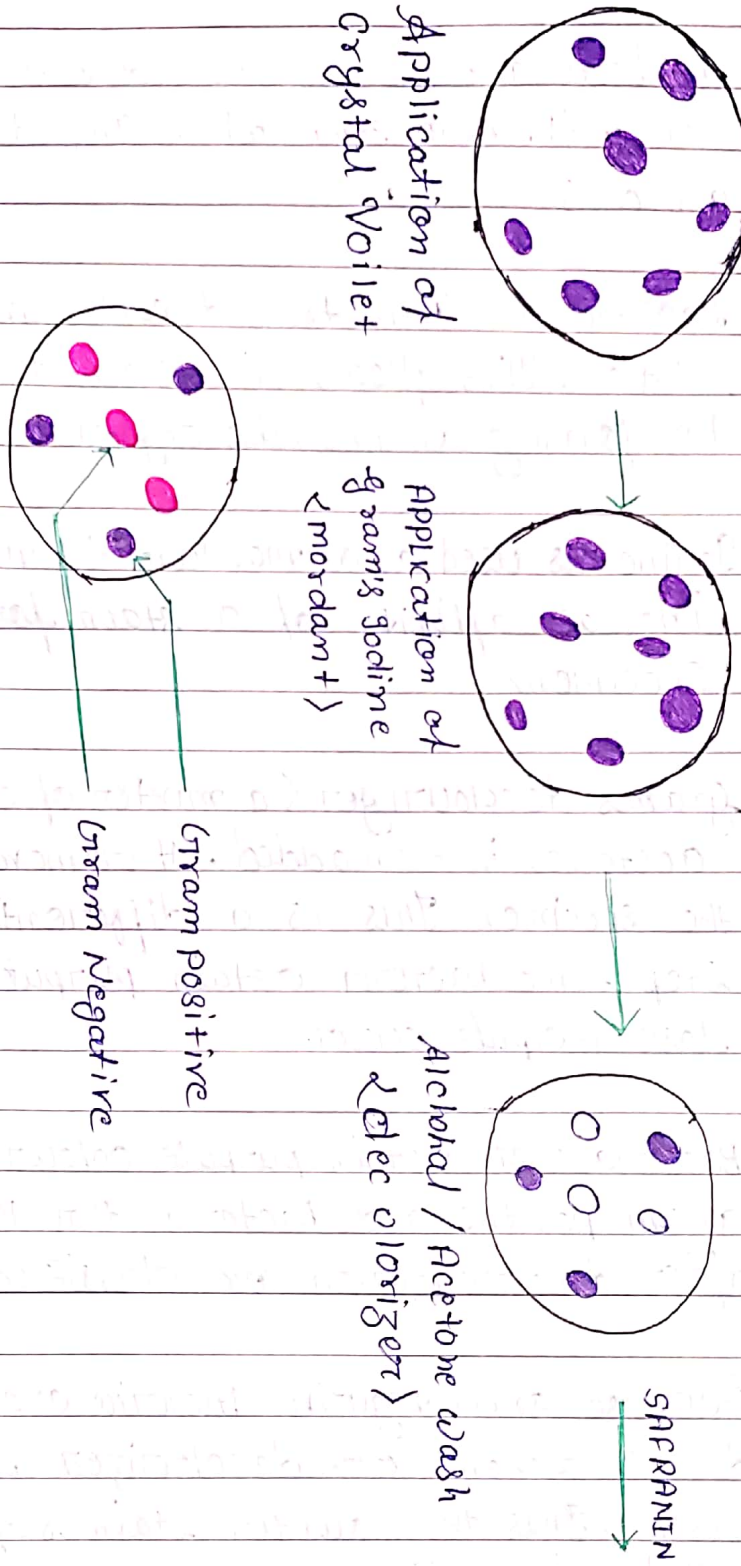
→ Iodine is used as a mordant (a mordant is used to increase affinity of a stain for a biological specimen)

→ Gram's decolourizer (a mixter of ethyl alchohal and acetone) is then added. It removes the stain from the specimen. This is a differential step. After this step some bacteria retain purpute colour while some lose purpule colour.

→ Bacteria that retain purpule colour are classificed as gram positive and bacteria that lose purpule colour after decolourization are classified as gram negative.

→ Because gram negative bacteria are colourless after the treatment with decolourizer. they are no longer visible. Thus the counter stain safranin (also a basic dye) is applied.

→ Gram negative bacteria that are noco colourless become directly stained by Safranin. Thus gram positive appear purpule. while gram negative appear Red or Pink.



★ Acid Fast Stain →

The acid fast stain is a differential stain used to identify the acid fast organism such as genus mycrobacterium.

→ Acid fast procedure involve heating of bacteria with the mixture of acid fuchsin and phenol (also known as Ziehl-Neelsen stain).

→ The presence of Phenol and heat treatment help the stain to penetrate the cell wall.

→ Once basic fuchsin has penetrated cell wall, acid fast cells are not easily decolourized by acid-alchohal treatment and hence remain Red.

→ It occurs due to the presence of a large ~~number~~ amount of Mycolic acid. (A branched chain hydroxy fatty acid)

→ Non acid fast bacteria are decolorized by acid alchohal

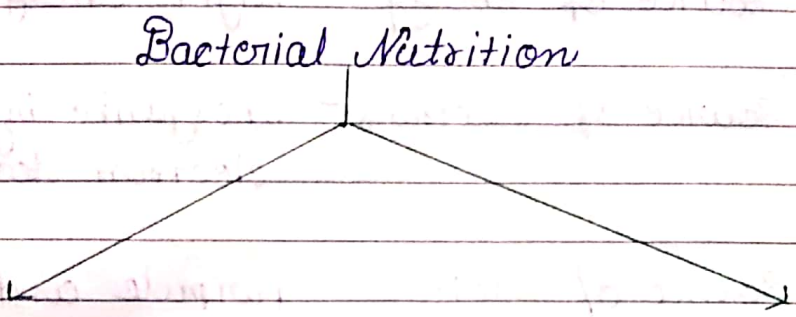
→ Because non acid fast bacteria are colourless after the treatment with decolourization, they are no longer visible.

→ Finally the counter stain Methylene blue is applied.

→ Methylene blue change the colour of non acid fast bacteria from colourless to blue.

☆ Bacterial Nutrition

* Nutrients are substances used in biosynthesis and energy production and therefore are required for bacterial growth.



Macronutrients
↓
Needed in relatively large amounts
↓
Example - C, O, H, N
S, P, K, Ca, Mg,
Fe

Micronutrients
↓
Needed in very small amount
↓
Example - Mn, Zn, Co,
Mo, Ni, Cu

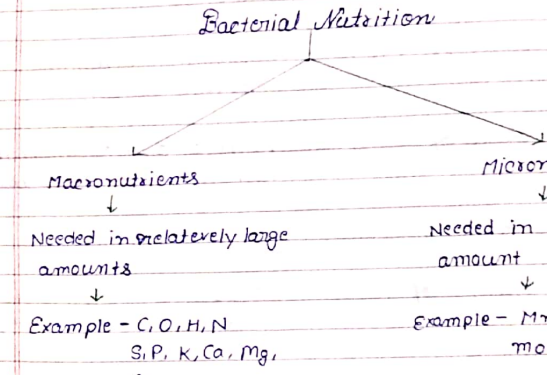
☆ Major Nutritional type of Bacteria :-

⇒ on the basis of source of carbon, energy and hydroge electrons acceptor / donar bacteria can be categorised into following types:

⇒ Based on the primary source of carbon, energy and ~~energy~~ electron, bacteria are placed in four major nutritional classes:

☆ Bacterial Nutrition

* Nutrients are substances used in biosynthesis and energy production and therefore are required for bacterial growth.



☆ Major Nutritional type of Bacteria :-

⇒ On the basis of source of carbon, energy and hydrogen electrons acceptor / donor bacteria can be categorised into following types:

⇒ Based on the primary source of carbon, energy and ~~energy~~ electron, bacteria are placed in four major nutritional classes:

i) Photolithotropic autotrophy
(Photolithoautotrophy)

→ Source of Carbon - Carbon dioxide

→ Source of energy - Light energy.

→ Source of electrons - Inorganic hydrogent electron donar

→ Source of bacteria - Purpule and green sulphur bacteria, blue-green bacteria.

ii) Photoorganohetrotrophy

→ Source of Carbon - organic carbon source (CO₂ may be used)

→ Source of energy - Light energy

→ Sourc of electron - organic hydrogen/electron donour e.g. water.

→ Representative bacteria - purpule non-sulphur bacteria, green non sulphur bacteria.

iii) Chemolethotrophy

→ Source of carbon - CO_2

→ Source of energy - Chemical energy source.

→ Source of electron - Inorganic hydrogen / electronic donour

→ Representative bacteria - Sulphur oxidising bacteria hydrogen bacteria, nitrifying bacteria.

Inorganic electron donour	groups	Representative groups
$NH_3 \xrightarrow{O_2} NO_2^-$	Ammonia oxidiser	Nitrosomonas europaea
$NO_2^- \xrightarrow{O_2} NO_3^-$	Nitrate oxidiser	Nitrobactor inogradskyi
$So, S_2O_3^{2-} \xrightarrow{O_2} SO_4^{2-}$	Sulphur oxidiser	Thiobacillus thio oxidance
$Fe^{2+} \xrightarrow{O_2} Fe^{3+}$	Iron bacteria	Thiobacillus ferroxidance
$H_2 \xrightarrow{O_2} H_2O$	H_2 Producer	Alcaligenes eutrophus
$CO \xrightarrow{O_2} CO_2$	Carboxido bacteria	Pseudomonas carboxydovorance

iv) Chemoorganoheterotrophy

→ Source of carbon - organic carbon source

→ Source of energy - Chemical energy source (organic source)

→ Source of electrons - organic hydrogen/ electron donar

→ Representative bacteria - Most non-photosynthetic bacteria.

★ Culture Media

* Culture media is necessary to provide the appropriate biochemical and biophysical environment.

* A culture media is a solid or liquid media that contains all the nutrients the microorganisms required for growth.

* It is used to grow, Transport and store microorganism.

* culture media are employed in the isolation and maintanance of pure culture of bacteria and are also used for the identification of bacteria according to their biochemical and

physiological properties.

* Culture media can be classified on the basis of physical nature (liquid, semi-solid, solid), chemical composition (synthetic and complex) and functional type (supportive, enriched, selective and differential).

1) Minimal media -

Those that contain the minimal nutrients possible for growth of wild type organism. It typically contains a carbon source which may be sugar such as glucose, various inorganic salts and water.

2) Supplementary media -

A type of minimal media that also contain a single selected agent usually an amino acid or a sugar for the culturing of specific auxotroph.

3) Synthetic and complex media -

=> Synthetic - A synthetic medium is chemically defined medium in which the exact chemical composition is known.

=> Complex - A complex is an undefined medium in which the exact chemical constituents of the medium is not known.

example → Nutrient broth, Tryptic soy broth and mac conkey agar.

4) Enriched media –

An enriched medium contains some components that permits the growth of specific type of species of bacteria usually they alone can utilise the component from their environment.

⇒ An enriched medium may have ~~selective~~ features

Example – Blood agar is an example of enriched media because it encourages the growth of many fastidious bacteria

5) Selective media –

Selective media is a one which has a components or prevent the growth of certain type of species of bacteria and /or promote the growth of desired species

⇒ For example →

i) Bile stats and crystal voiled favoured the growth of gram negative bacteria by inhibiting the growth of gram positive bacteria.

ii) Eosin methylene blue agar, mac conkey agar and manitol salt agar and commonly used as selective media.

6) Differential media -

A culture media is described as differential media if it allows to investigate or to distinguish between different types of bacteria based on some observable trait in their pattern of growth on the medium

=> Blood agar is an example of differential media and is used to distinguish between hemolytic and non-hemolytic bacteria.

=> Example - Eosin methylene blue agar. MacConkey agar, Manitol salt agar are also used as differential media.

7) Pure culture - A pure culture is ~~that~~ one that contains only a single kind of microbial population growth from a single cell.

A pure culture is usually derived from a mixed culture (containing many species) by methods that separate the individual cells so that when they multiply each will form individually distinct colony which may that be used to establish new cultures with the assurance that only one type of organism will be present.

A Difference between gram negative and gram positive bacteria.

	Property	Gram negative Bacteria	Gram Positive Bacteria
①	Cell wall	2-7 nm	20-80 nm
②	Techoic acid	Absent	Present
③	Plasmic Space	Prominent	Negligible
④	Outer membrane	Present	Absent
⑤	Motility	Motile or non-motile	Mostly non motile
⑥	Appandages	usually bear appandages like pili or fimbrae	usually absent
⑦	Flagellar structure	four rings in basal body	Two rings in basal body
⑧	Toxin Produced	endotoxin and exotoxin	Exotoxin
⑨	Endospore	Can't form endospore	formation of endospore take place

4.)	outer membrane	present	Absent
5.)	Motility	Motile or non-motile usually bear appendages like pilli or fimbrae	Mostly non-motile usually absent
6.)	Appendages		
7.)	Flagellar structure	Four rings in basal body	Two rings in basal body
8.)	Toxin produced	Endotoxin and exotoxin	Exotoxin
9.)	Endospore	Cannot form endospore	Formation of endospore takes place.

☆. # Water microbiology

☆. ## Types of Water - And its pollutant.

Natural Water is commonly group into four well masked classes.

a) Atmospheric Water
b) Surface Water
c) Stored Water
d) Ground Water

a) Atmospheric Water

- Rain and Snow are included in the atmospheric Water.
- Microorganisms in the form of dormant propagules and dust particles remain suspended in Water and snow considerable no of bacteria can be isolated from rain Water after heavy rain or snow fall the dust particles and bacteria are washed from the atmospheric therefore, atmospheric for Sometimes remains free from Microorganisms.

b) Surface Water

- The Water present on earth surface is known as surface Water.
- It is found in the form of several Water bodies such as rivers streams. Ocean, lakes etc.
- Total microbial number depends on microbial population of soil types of soil, types of organic material present in the soil and also types of Mos and their activities.

c) stored Water

Date
Page

- The stagnant land Water present in ponds, lakes etc are called stored Water.

- During storage in general the no. of microorganisms gets reduced their it establish to some extinct the purity and stability. However in stored Water the microorganisms are affected by several foundation such as sedimentation, ultra violet light, temperature, osmatic, food supply and activities of other Mos as Well.

d) Ground Water

- Soil consists of particles of varifying size therefore, soil pore size varies of the percentage, space and size of power regulate the quantity of rain Water than can be soaked and held in soil.

☆. Water microorganisms –

- A large no. of Mos both saprophytes and pathogens are found in Water which fall under the group bacteria, fungi, algae, protozoa and nematods several animals, viruses are also transmitted to Water.

- The majority of bacteria found in

Water belongs to groups alginomonas chromogenic rods (xanthomonas etc), coliform groups (E. coli).

☆. Microbial analysis of Water purity

- The natural water bodies such as lakes streams rivers contains sufficient amounts of nutrients the support the ground of Mos. However there are different ways by which microorganisms enter in water supply for eg:- broken sieven lines congested centres in approach private treatment etc in addition lack of awareness among people suffering from communicable also discharge pathogenic microbes in water through their exert for eg:- amboeaio dysentry typhoid fever.

☆. The faced coliform

- On the basis of microbial examination of water its probality (Suitability for drinking water may be as counted intestinal bacteria present in water generally do not survive in aquatic environment due to physiological stress but if entered human being system

in the meanwhile the cause serious problem the characteristic group of intestinal bacteria are the coliform.

- coliform bacteria live in soil, or vegetation and in the gastrointestinal tract of animal.
- coliform are not a single type of bacteria, but a grouping of bacteria the includes many strains such as E. coli.
- Coliform enter water supplies from the direct disposal of waste into streams or lakes from run off from wooded areas, pastures sewage plants or ground water.
- Total coliforms are the standard by which microbial contamination is measured.

☆. Non - coliform bacteria

- Non - coliforms bacteria from example Salmonella & shigella.
- Non - coliform don't age lactose and are pathogenic (genuine pathogens). They are subcategories of the family Enterobacteriaceae.

☆. Test for coliforms - are following

1.) Sanitary test for coliforms -

a) presumptive test
b) Confirmed test

2.) The most probable number of (MPN of coliforms) -

a) The membrane filter technique.

3.) The defined substrate test

4.) IMVIC test.

☆. What is Water microbiology

Water microbiology is the scientific discipline that is concerned with the study of all biological aspects of the microorganisms (bacteria, archae, viruses, fungi, parasites and protozoa) that exist in water. This is also known as marine microbioly, which is a subdiscipline of environmental microbiology.

☆ Composition of sewage

The composition of sewage mainly depends upon per capita consumption of water and various from place to place and season to season.

two types of sewage composition.

Sewage

1.> chemical composition -

chemically. the sewage consists of approximately 99% water and 1% inorganic and organic matter in suspended and soluble forms.

2.> Microbial composition -

The microbial population per milileter of sewage may vary from a few lacs to several millions. Various types of Mos viz. micro-fungi, bacteria and protozoa collectively called sewage fungus are known to grow profusely in sewage.

☆. purification or disposal of Water (Water-treatment)

The methods and technique of purification of Water are following. There are several methods for purification of water, the use of which depends on amounts and quality of Water.

eg:- purification of water required for a single house holds differs from that of a town or city.

Secondary.

purification of Water is essential before its consumption so that diseases cycle of pathogenic Mos can

be broken. Thus purification is done with the prospect of making it satisfactory in appearance although and free from pathogens these.

Hence cities which are used for the purification of drinking water in municipal supplies.

Sedimentation, filteration, and fision factor (Kables 1962) the supplies of water purification at manipal supply are given.

☆. Sedimentation ⇒ 1st method

- Sedimentation is done when water consists of large size organise materials such as leaves and gravels which have turn of form the soil suspended particles settle down depending on their size weight and condition of the stored water sedimentation is done is large rever various or in restricted area of sebling tank.

- The rate of sedimentation in enhanced by adding alum, ions, salt, colloid silicates which acts as coagulants.

The

Suspended materials and microorganisms are entrapped by coagulants and settle down rapidly this procedure is called Coagulation or flocculation the microorganisms remain viable for sometime, thus sedimentation provide particle reduction of micro-organisms in water due to their selling down to bottle but does not sterlise the polluted water.

☆. Filtration ⇒ 2nd method

- The second step of purification after sedimentation the water is further purified by passing to filtration unit it is the effective means to removing batches that contaminates from drinking water.
- The filtration apparatus consist each box which containg 3 and which does the filtering gravel which keeps the sand from getting out and understand where the filtered water exists slow through filter is reversed and the sand and particles are suspended.
- These are two types of sand filter which are used in water purification such as slow sand filtration and

Rapid Sand Filteration.

1. Slow Sand Filter
2. Rapid Sand Filter

A. Disinfection ⇒ 3rd method

Some of bacteria has through filter, even after filteration which must be killed because contamination of water. Therefore disinfication of public water supply needs to be done disinfication is the final step of water purification solution of sodium hypochloride are used in small town but in recent years chlorination public water supply has become popular.

☆. Food Microbiology

- Food is the important source from existance of all living organisms food provides nourishment and energy to the living organisms. All metabolic reactions occures in the presence of various proteins, enzymes, vitamins, lipids and these substances comes from food.

- Food supply consists basically of plants

and animals or products derived from them. These food supply can contain various microorganisms so the interaction of MOs with food is known as Food microbiology.

- These microorganisms use food supply as a source of nutrients for these growth. There are two possibilities during these -

a) Result in spoilage of food.
b) These interaction between microorganisms and food give beneficial to human.

- When interaction occures between MOs and food then microorganisms utilise nutrient of the food it involves changes in the food compound or synthesis of new compound that cause spoilage of the food of or produced enzymatic changes during breakdown a food product.

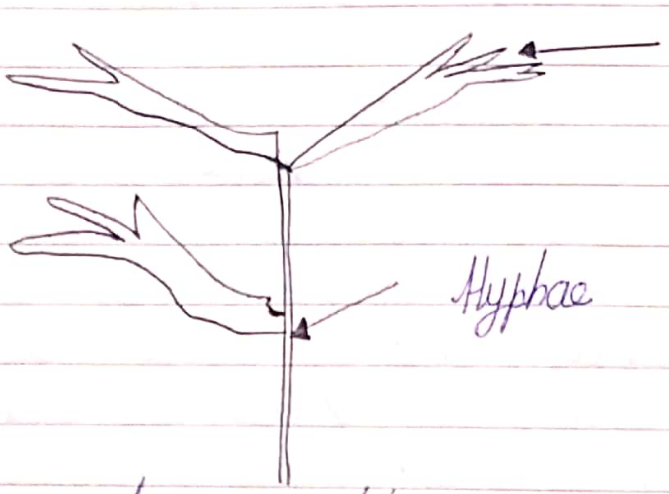
- To prevent spoilage of food from MOs. We should minimized the content bet^n MOs and food, eliminate microorganisms food and preservation on the food.

Q. Important microorganisms in food microbiology ?

→ There are various microorganisms that interact with food. They are - Moulds, yeasts, bacteria and viruses.

Moulds

i) These are eukaryotic organisms.
ii) They are multicellular, Non-motile, filamentous and branched microorganisms.
iii) These all composed of large no. of filaments called hyphae which are aggregated and called mycelium.



iv) Reproduction of moulds occurs from spore formation. eg:- Moulds - penicillium species.

Yeasts

i) These are eukaryotic cells or organisms
ii) They are unicellular, oval, spherical or elongated.

iii) They are non-motile microorganisms.
iv) Reproduction by fragmentation process and can see budding formation. eg:- Saccharomyces cerevisiae (scientific name)

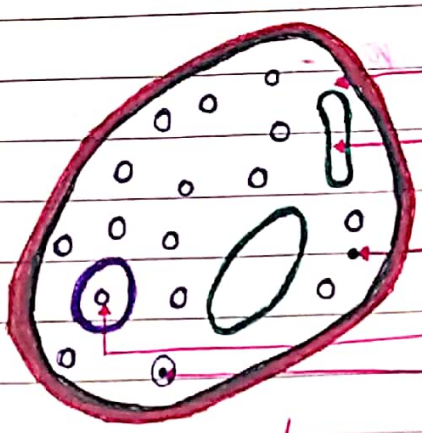


Fig:- yeast

Bacteria

i) These are prokaryotic microorganisms.
ii) They are unicellular and having three morphological forms - spherical (cocci) - eg:- streptococcus species (spp). Bacilli (rod) - eg:- Bacillus species. Curved (comma) - eg:- Vibrio cholerae.

iii) Bacteria can be motile or non-motile.
iv) Thus cytoplasmic materials are enclosed in a rigid wall on the surface and a membrane within the wall.
v) The organel doesn't enclosed in a seperate membrane that is all organelle lies in the cytoplasm without membrane.

vi) bacteria grouped age -

a) Gram negative - contain outer membrane which is composed of lyco-polysaccharide (due to many enzymes, antibiotics, salts etc.)

b) Gram positive - They have thick wall composed of several layers of mucopeptide and taichoic acids.

VIRUSES

i) These are non-cellular microorganisms.

ii) Most important are bacteriophase (bacterial viruses).

iii) Viruses contain nucleic acid (DNA & RNA) and protein.

FOOD SPOILAGE

- Any changes in the visual, smell and texture of food that makes it unacceptable for consumption each known as food spoilage.
- Most serious economic problem in food processing industry.
- Food are organic substance that provide nutrients from the growth of various microbes these microbes attack on food

by various process such attack is harmful to the quality of food.

☆. Causes of spoilage

a) Growth and activity of Mos.
b) Due to insects.
c) Action of enzymes to the plants & animals food.
d) Due to chemical reactions.
e) Due to physical changes of food like freezing, burning, drying etc.

☆. Types of food spoilage :

i) physical spoilage- Dehydration of vegetable.

ii) chemical spoilage oxidation of fat, browing of fruits and vegetables.

iii) Microbial spoilage Due to growth of microorganisms enzymes production by microorganisms.

☆. Important microorganism in food microbiology

i) Moulds genera

a) Genus aspergillus

- These are widely distributed and contain many species important in food microbiology.
- shaped hyphae and produce asexual spores or conidia.
- xerophilic
  eg:- Aspergillus flavus
- Strains are used in food processing.

b) Genus geotrichum

- Septate hyphae and produce spores.
- Grow and forming a yeast like colony as well as grow on dairy product.

c) Genus Mucor

- Widely distributed.
- Non-septate hyphae and produce sporangio phores.
- Some species are used in food fermentation and other can cause spoilage of vegetables.

d) Genus rhizophus

- Widely distributed & contains many species.

- Septate hyphae and conidia, phoma.
- Some species can cause spoilage in food. Vegetables, grains, bread etc.
- They can also produce mayco toxin.

ii) Important yeast genera

a) Genus Saccharomyces

- Cells may be round oval and elongated.
- Reproduction is by budding or by ascospore formation.
- These species are used many food industries eg :- bread manufacturing, wines, alcohol etc.
- Genus Saccharomycee lactic is important in milk and milk products because they are common spoilage Mos.

b) Genus Torulopsis

- General spoilage yeast.
- They spoils a variety of food products like beer, milk products, fruits Juices s and some refrizerated fruits.

c) Genus Candida

- Many spoil foods high acids, salt and sugar form pellicle on the surface of

liquids.

- Some species can cause rancidity in butter and dairy products.
- Can form pseudo hyphae & and true hyphae with many budding cells.

d) Genus Rhodotorula

- They cause (red, pink or yellow yeast) dies colouration on food such as in meat and fish etc.

iii) Important bacteria genera

a) Genus bacillus

- Different species may be mesophilic and thermophilic.
- Spores produced by these bacteria are generally heat registered.
- Some species may cause food born disease and food spoilage canned products.
- The soil is an these source species.

b) Genus clostridium

- Rod shaped cells, anaerobic and form endospores.
- Founding soil marine Sediments, animals

& plant products.
- Some are pathogen while others are important in food spoilage.
- Some species cause stormy fermented of food (discruption of curd in milk).

c) Genus eschexichia

- Found in faeces, gram negative rod cells isolated form the intestinal tracts of warm bluded animals.
- E. coli used as an indicator of sanitation in the coliform in faced coliform group.
- Many strains are non pathogenic but some can be pathogenic to humans and animals (food burn disease).

d) Genus lactobacillus

- Rod shaped facultative and anaerobes. non- motile, mesophilic.
- Can be homo and hetero lactic fermentors.
- Found in plant sources, milk, meat and fishes.
- uses - (i) food bioprocessing - eg:- L. lactis
  (ii) probiotics - eg:- L. acidophinus

- Spoilage - They causes like Wine a beer production, cheese, making and canned survive pasteurization.

e) Genus pseudomonas

- These are gram and aerobic, rod-shaped motile bacteria.
- Important in feace & meat spoilage.

f) Genus staphylococcus

- They are frequently involved in food burn disease.
- Its gives yellow to orange growth.
- Many species causes food poisioning.

☆ Nitrogen fixing of microbes in agriculture

- Nitrogen is very important compound essential for all living organisms.
- These are approximate 78% N_2 present in our atmosphere but we can't take it directly.
- Microorganisms are the very important

source that provided nitrogen easily to all living organisms.

- atmospheric N_2 - fixed by MOs fixedly into nitrate then nitrite.
- These are so many microorganisms these are - azotobactor, azospixillum and azolla species Rhizobium.
- N_2 - fixing bacteria are -ve and +ve symbiatic soil & plant accociated bacteria.
- N_2 - fixing bacteria such as azotobactor, azospixillum are well known for they are ability to development.
- Many of these bacteria can produce and spixit and these cultures.

Azospixillum

- azospixillum species belong to the facultative indophytic, diazotroph species. which colonies the surface and the interior of foods. These kind of association is considered as the starting point of most biological N_2 - fixation process with non legume plants.

N_2 - fixing organisms such as azospirillum directly benefits plants improving soil and root development increasing the rate of water and mineral uptake by roots.

Azotobactor

- azotobactor is an obligate aerobes. as well as it can grow under low O_2 concentration.
- The ecological distribute these bacteria is mostly in soil
- Bacteria of the genus azospirillum are found mostly in soil or tropical and subtropical and temperate regions
- azotobactor develop in close relationship with the root of various wild & agricultural plant.

Azolla species

- azolla species comes under several floating water ferns (fungi) species.
- They are present mostly in tropical and temperate ecosystem.

- It has the ability to fix atmospheric Nitrogen through symbiosis with blue-green alga (Nostac & anabena).
- azolla species are important source of N_2 for Weightland rice.
- The contribution of nitrogen for azolla species to Weightland rice plants has been found to be maximum when incorporated into the soil green manure.

eg:

Biological nitrogen - fixation

There are two types of N_2 fixing bacteria -

(1) Symbiotic

- Fixation of free nitrogen by mos in soil living symbiotically in the plants.
- These are three types -

(i) Nodule formation in leguminus plants - eg- by rhizobium species.

(ii) Nodule formation in non- leguminus

plant. eg- by rhizobium species.

(iii) Non- Nodulation. eg-

(a) Lichens - by cyanobacteria.

(b) Anthoceros - by Nostoc.

(c) azolla - by anabena azollae

(d) cycas - by Nostoc & anabena

(2) Non- symbiotic

- Fixation carried by free living M_0, aerobic, anaerobic and blue-green algae.
- special type of N_2- fixing bacteria are-

(a) Free- living aerobic - eg- azotobacter, beijerendia.

(b) Free- living anaerobic -eg- colostridium.

(c) Free- living photosynthetic. eg- chlorobium rhodopseudomonas.

(d) Free - living chemosynthetic · eg - desulfovibrio, thiobacillus.

- Fungi - Free living fungi · eg - yeast & pillutesia

- Blue - green alga -

(a) unicellular - eg - Gloeothece, symechococcus.

(b) Filamentous - (non - heterocyst) - eg - occilla - toxia.

(c) Filamentous - (heterocyst) - eg - tolypothrix, Nostac, anabeena.