UNIT -2
STRUCTURE OF BACTERIA, VIRUSES AND PURE CULTURE CONCEPT

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INTRODUCTION:

Bacteria(eubacteria) are microscopic living organisms believed to be first primitive organisms on our planet. They are typical prokaryotes and also possess characters resembling both plants and animals.

GENERAL CHARACTERS:

1. Prokaryotes lacks intra cellular bounded organelles.
2. Prokaryotic cells are always bounded by chemically complex cell wall which differ in gram positive and gram negative bacteria.
3. Next to cell wall there is a plasma membrane which lies inside the cell wall separated by periplasmic space.

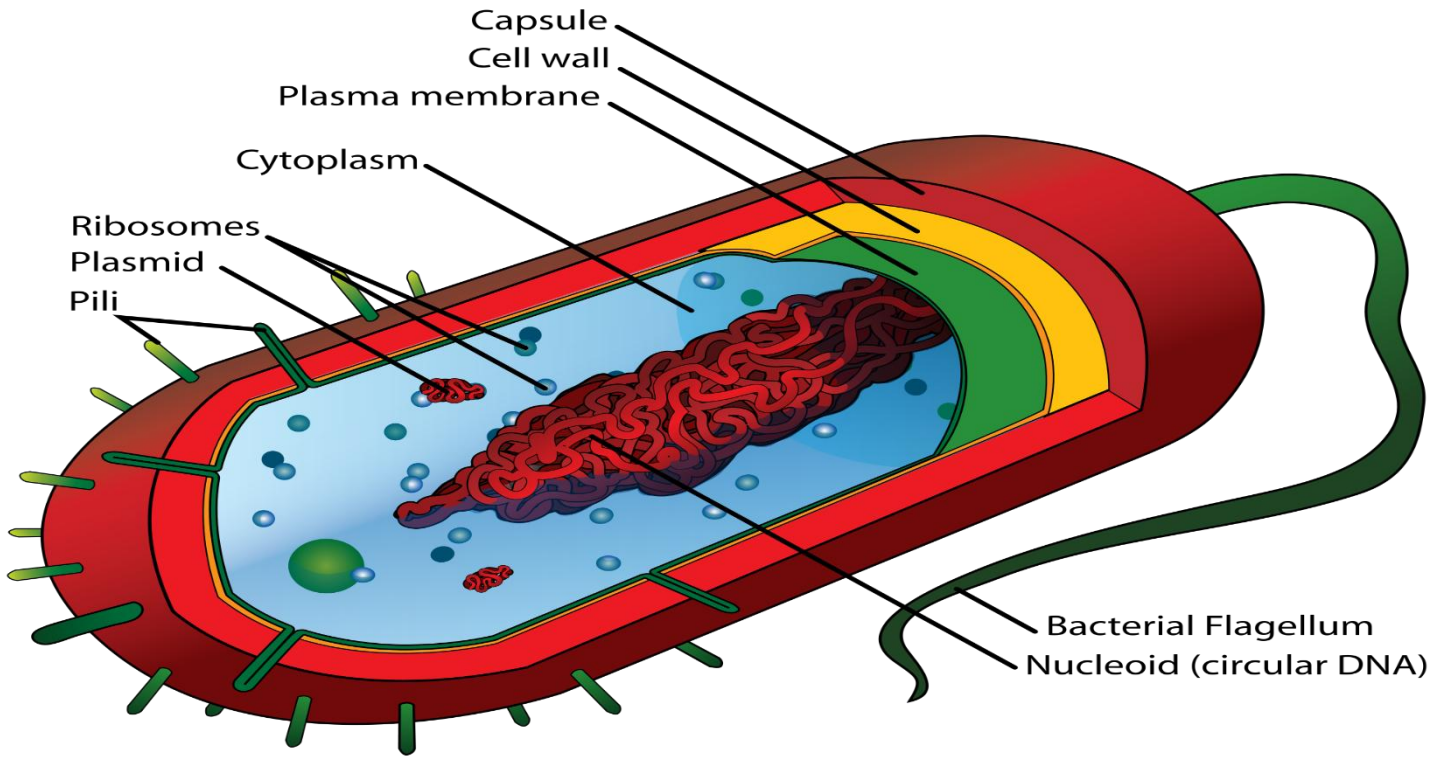
4. The plasma membrane is invaginated inside to form “Mesosomes”.

5. The genetic material is located in nucleoid which is not separated from the cytoplasm by membranes.

6.Ribosomes and inclusion bodies are scattered in the cytoplasm matrix.

7. Flagella and fimbriae are helping in locomotion.

8. The cell wall surrounded by external layer called capsules and slime layers.



1. FLAGELLA :

- Bacteria(prokaryotes) have the ability to move by means of appendages called flagella. They are used in locomotion.
- Flagellum is a hair like appendages that protrudes through the cell wall and originates from basal boy just beneath the cell membrane in the cytoplasm.

Based upon the number and position of flagella, bacteria are further classified into various types they are:

MONOTRICHOUS: Bacteria are with one flagellum e.g: Pseudomonas aureginosa.

LOPHOTRICHOUS: Bacteria with tuft of flagella at one end. e.g:Pseudomonas fluorescence.

AMPHITRICHOUS :Bacteria with tuft of flagella at both ends.e.g :Alcaligens.

PERITRICHOUS : Flagella are present all over the bacteria. e.g : Salmonella typhi

STRUCTURE OF FLAGELLA:

Each flagellum consists of three parts

- Basal body
- Hook
- Shaft

BASAL BODY:

- It consists of two sets of rings connecte by a rod.
- Each set has two rings and all together there are four rings.
- The basal body is different from gram positive and gram negative bacteria.
- Four rings are named as M= membrane ring, S= super membrane ring, P= peptidoglycan ring and L= lipopolysaccharide ring. from the inner to outside.

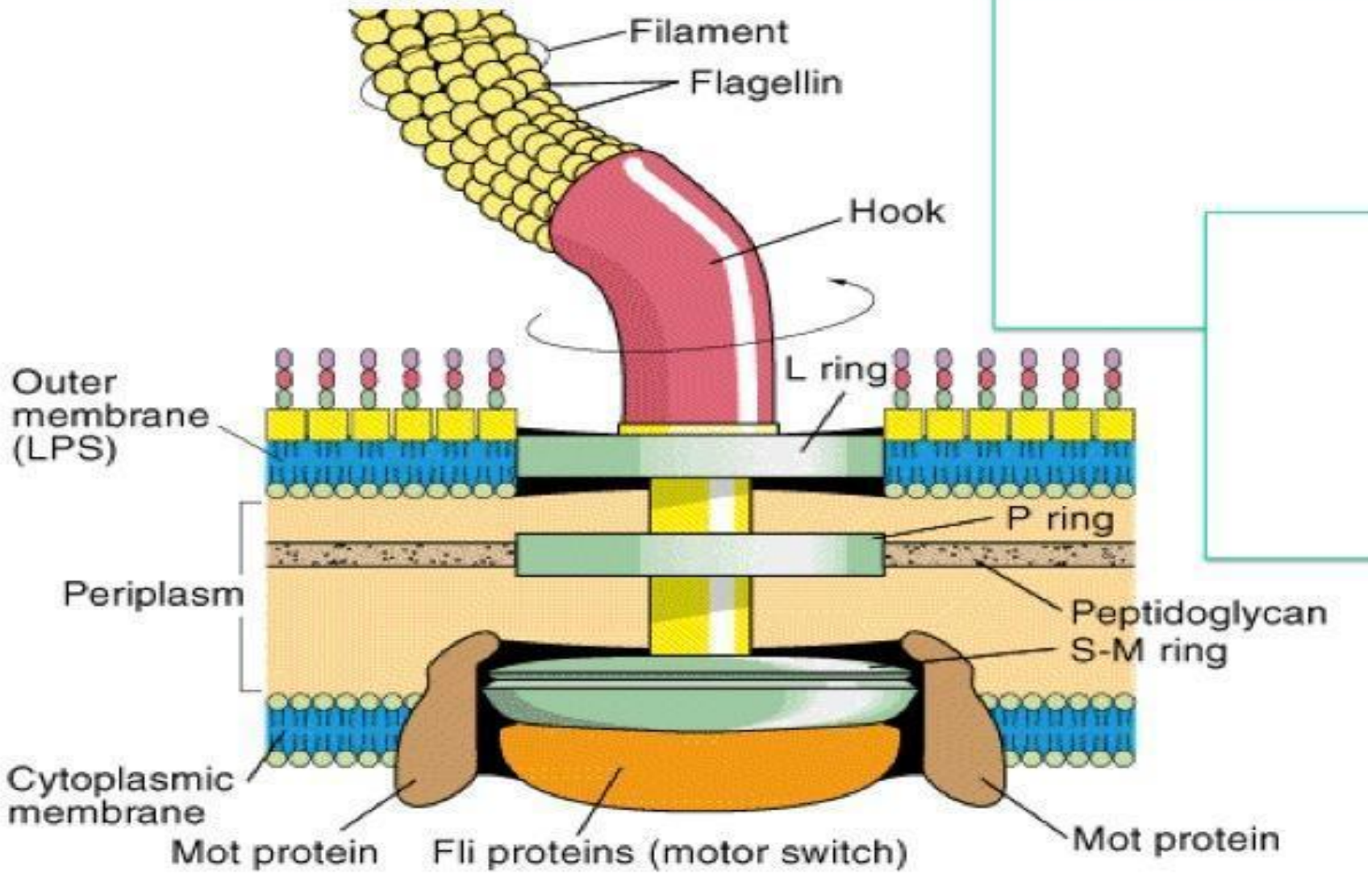
- In gram negative bacteria E.Coli four rings arre present connected to a central rod.
- The outer L and P rings remain embeded in the lipopolysaccharide and peptidoglycan layers.
- The inner S and M rings are located in the cytoplasmic membrane. In the gram positive bacteria L and P rings are absent.

HOOK:

- It is 45nm length. It is short, curved wider than the filament.
- It connects the basal body with the shaft.
- It is made up of protein subunits called as “protomers”.

FILAMENT:

- The outermost structure is the filament. It is about 120-200 A in diameter composed of aprotein called as “flagellin” with a molecular weight of 30,000 to 60,000KD and this is attached to the basal granule with the aid of hook.
- The length of the flagellum is about 20-30 um and 15-20nm in thickness.
- The bacterial flagellum consists of eleven fibrils, which are arranged hellically around an axil cylinder.


Filament
Flagellin
Hook
L ring
Outer membrane (LPS)
P ring
Periplasm
Peptidoglycan
S-M ring
Cytoplasmic membrane
Mot protein
Fli proteins (motor switch)
Mot protein

LOCOMOTION: There are three types of movement in bacteria..

Flagellar movement:

- The bacterial flagella is semi rigid helical rortor that moves the cell from basal body either clock wise or anti clockwise.
- The helical waves are generated from base to the tip of the flagellum.
- The basal body acts as motor and causes rotation.

Gliding movement:

- Bacteria like cyanobacteria and mycoplasma show gliding movement when come in contact with the solid surface(wood, bark, and stem)

Spoirochaetial movement:

- The spirochaetes show different types of movements such as spinning, free swimming, flexing and creeping as they are flexible and helical bacteria which lack flagella.
- They have periplasmic flagella or axil fibrils or endoflagella.

2.PILI (FIMBRIAE)

- Pili are hair like non flagellar appendages present on the suraface of gram negative bacteria.
- They are smaller, shorter and more numerous than flagella.The term pili was introduced by “Brinton” (1950) and fimbriae by “Duguid”.
- Pili can be observed only under electron microscope.
- Pili are made up of protein subunits called “pilin”.
- The production of pilin is controlled by thegenes located on plasmids and consists of about 163 aminoacids.

according to the function, pili are of two types

- *COMMON PILI*: Which acts to adhere the cell to surfaces.
- SEX PILI: Helps in joining t other bacterial cells for transfer of genome.

FUNCTIONS OF PILI:

- Possessantigenic property.
- Fimbriae agglutinates with blood cells.
- Fimbriae affects metabolic activity.
- Sex pili transfer genome by the help of conjugation tubeone cell to another.
- Some pili are used for attachment to pathogenic bacteria.

3. CAPSULE/SLIME LAYER:

- The capsule refers to the layer attached around the cell wall,whike slime layer layer refers to the copious, loosely associated structure around the cell wall.
- Capsules can be obsereved with compound microscope by staining with negative stains like indian ink,nigrosin etc.
- Capsules are composed of polysaccharides (Streptococcus, Xanthomonas) or polysaccharides (Bacillus subtilis, Bacillus anthracis)

FUNCTIONS OF CAPSULES:

- Capsules have virulence. capsules protect pathogenic bacteria from phagocytosis.
- Capsule protects bacteria aginst dessication as it contains more water.
- They exclude bacterial viruses and most hydrophobic toxic materials such as detergents.
- It helps in attaching various surfaces such as streams, plant roots, teeth,tissues, and other bacteria.
- These are species specific.
- It serves as a buffer between cell and external environment.

4.CELL WALL:

- It is the outer layer that sourrounds cytoplasmic contents and is present beneath the capsule or slime layer in all bacteria except mycoplasma.
- Cell wall is tough and rigid structure protects from osmotic pressure and gives shape to the cell.
- The thickness of the cell wall ranges from 100-250nm. The cell wall was introduced by Horne and Salton.
- Based on the cell wall structure bacteria are divided into two groups Gram positive and Gram negative.
- The backbone of bacterial cell wall is peptidoglycan/murien/muramic acid/mucopeptide. This is absent in archaebacteria.
- Based on cell wall, Christian Gram developed gram staining in 1884, bacteria divided inti gram positive and negative.
- The cell wall is composed of peptidoglycan, diaminopimilic acid(DPA), muramic acid and teichoic acid.

- Peptidoglycan consists of two prts of glycan, sugar and a peptide portion.
- Glycan portion is made up of N- acetyl glucosamine (NAG) and N- acetyl muramic acid (NAM) bounded by Beta 1,4 linkages.
- Peptide portion is short chain composed of four amino acids(L-alanine, D-glutamine,L-lysine/diaminopimilic acid and D-alanine) connected with each other by peptide linkages called tetrapeptide chain which are interlinked by cross linkage between carboxyl group(C) and amino group(NH) of amino acid in tetrapeptide chain.

FUNCTIONS OF CELL WALL:

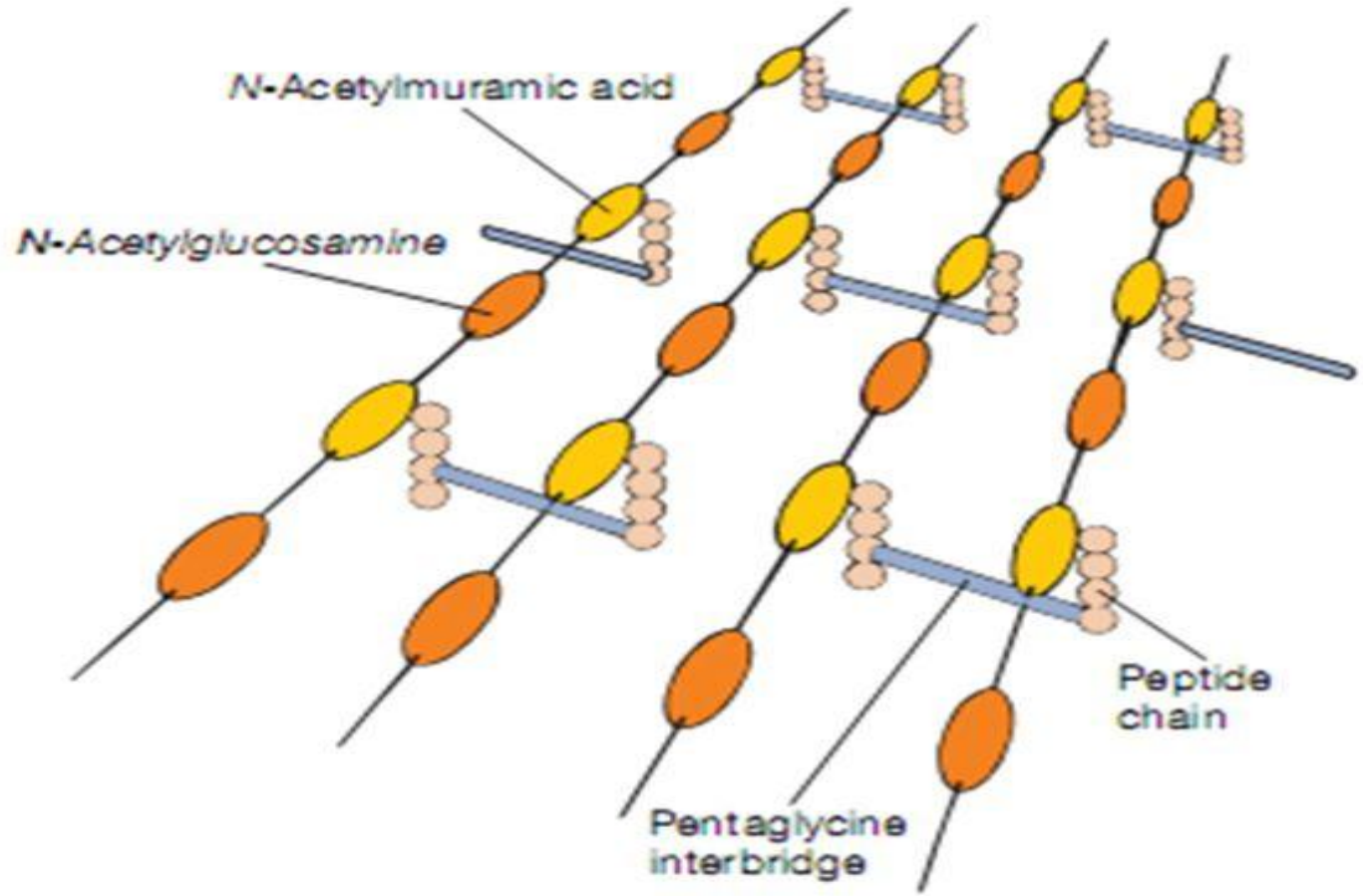
- Peptidoglycan provides structural integrity to the cell which makes the outer membrane rigid.
- It acts as protective barrier which permits molecules upto a particular size to penetrate.
- The matrix proteins act as receptors for various nutrients, colicins and bacteriophages.
- It is in location of respiration, photosynthesis,lipid synthesis and cell wall constituents.

- The most important component of cell wall is peptidoglycan(mucopeptide/murien/muramic acid)
- This component is present in both gram(+) and gram(-) bacteria.
- In gram positive bacteria 40 sheets of murien is present whereas in gram negative only two are present.
- Peptidoglycan is made of two important parts, a glycan portion and a peptide portion.
- It is structurally divide into 3 components i.e backbone, tetrapeptide side chain and peptide interbridge.

BACKBONE:

- The glycon or sugar portion of peptidoglycan forms the backbone.
- It is composed of alternatively repeating units of aminosugars N-acetylglucoamine (NAG) and N-acetylmuramic acid (NAM) linked to each other by beta 1,4 glycosidic bond.

Peptidoglycan Structure



TETRAPEPTIDE SIDE CHAIN :

- This compound of peptidoglycan is a short peptide and os composed of four amino acids includes L-alanine, D-glutamic acid, L-lysine and D-alanine.
- The tetapeptide chain sidechain os connected to carboxyl group of NAM residue.

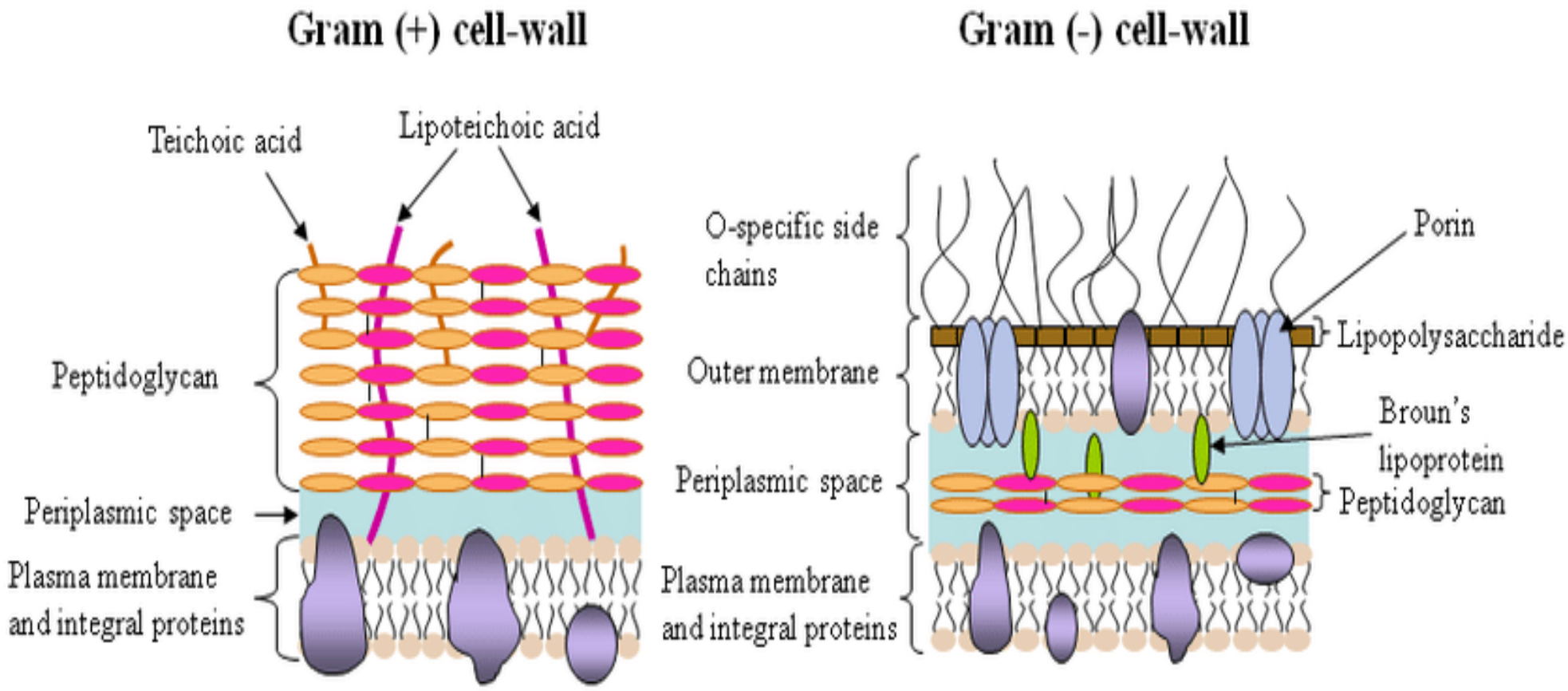
PEPTIDE INTERBRIDGE OR CROSS LINK:

- Two tetrapeptide side chain of two adjacent murien strands arelinked by cross links.

GRAM POSITIVE CELL WALL:

- It contains several layers of peptidglycan which are interconnected by side chains and cross bridges.
- Its thickness provides rigidity to the cell wall.
- Peptiglycan layer is thick than gram negative.
- Gram positive cell wall contains polymers containing phosphorous called " Teichoic acid" consist alcohol,phosphate and are flexible and hrdrophilic in nature.
- Teichoic acid plays imp role in growth of bacterial cell. Ex: Bacillus, Streptococcus,Clostridium.

- Techoic acid is a polymer of glycerol or rabtol, joined by phosphate groups.
- It is connected to either the peptidoglycan or to plasma membrane lipids.
- In the later case they are called as lipotechoic acid.



GRAM NEGATIVE CELL WALL:

- Gram -ve cell wall contins an outer membrane which is present ouside the peptidoglycan.

BRAUNS LIPOPROTEIN:

- It is the small lipoprotein covalently joined to the peptidoglycan and embeded in the outer membrane by its hydrophobic end .
- BL is linking the outer membrane and peptidoglycan.

OUTER MEMBRANE:

- Made up of 3 imp parts which consist the lipopolysaccharides(LPS)
- LPS consists of 1) Lipid A 2)Core polysaccharide 3) “O” side chain.

i) LIPID “A”:

- It containstwo sugar derivatives each with 3 fattyacids and a phosphate attached.
- It is embedediside the outer membrane and it makes the LPS to project ouside the surface.

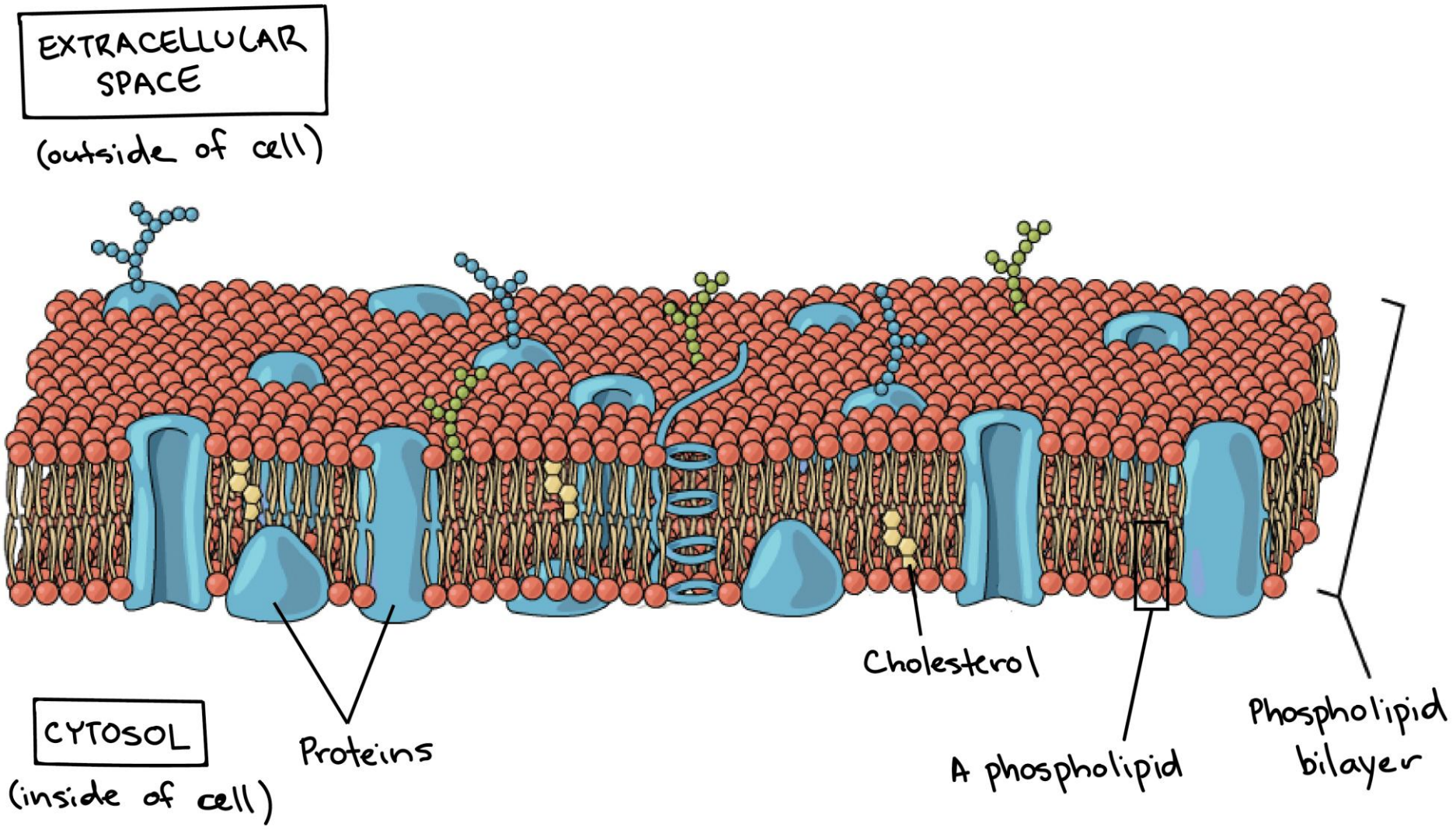
CORE POLYSACCHARIDE:

- It is joined to the lipid A. e.g:Gramnegative bacteria Salmonella which contain 10 different sugars with different structures.

“O” SIDE CHAIN:

- Also called as O- antigen. It is a polysaccharide chain extending outward from the core.
- It conatins many different sugars which differs between species.
- These O- side chains are readly recognised by thr host antibodies but the gram negative bacteriaprevent them by changing the nature of O- side chain.
- It also helps to protect the cell wall from the attack by interacting with the antibodies before they reach the outer membrane.

5.PLASMA MEMBRANE:



- It is made up of proteins and lipids with varying proportions according to fluid mosaic model.
- It is the external membrane of eukaryotic and prokaryotic cellwhich encompasses the cytoplasm.

Lipids:

- The lipids associated with plasma membrane are assymetrical.
- It is also called as amphipathic in nature.
- The amphipathic lipidds are phospholipids.
- The amphipathic lipids forms a bilayer at the top and bottom of plasma membrane.
- The polar ends present in the outsoide surface will interact with water and are called hydrophillic in nature.
- Where as the non polar ends are insoluble in water which are called hydrophobic in nature. The non polar ends are burried inside the cell.

PROTEINS:

- There are 2 types of proteins present in plasma membrane one is peripheralprotein and the other is integral protein.

PERIPHERAL PROTEINS:

.It is loosely connected to the membrane and can be easily removed.

.They are soluble in water and hydrophillic.

.It occupies 20-30% of total membrane protein.

INTEGRAL ROTEINS:

. It is firmly attachd to the plasma membrane, very hard to separate.

. The integral proteins are insoluble in water and hydrophobic.

. The integral protein occupy 70-80% of total protein.

NOTE: Proteins are also amphipathic in nature with hydrophobic regions inside and hydrophillic regions outside and thes proteinns can diffuse laterally around the suraface. They can move from one location to another but they cant rotate or flip-flop through lipid layer.

ALPHA HELIX:

- It stabilizes the plasma membrane and glycolipids are attached to the phospholipids within the membrane.
- Plasma membrane also contains pentacyclic sterol like structures called “Hoponoids”.It stabilizes plasma membrane.

PLASMA MEMBRANE:

FUNCTIONS:

- It helps to retain the cytoplasm especially in the cells without cell wall.
- It is selectively permeable particular ions and molecules in and out of the cell.
- It helps to transfer the nutrients inside the cell and excretion of waste products, protein secreations outside the cells.
- It acts as alocation for many metabolic processes like respiration, photosynthesis,synthesis of lipids and cell wall constituents.

- They also possess some special receptor molecules which helps to detect and respond to the chemicals in their sourroundings.
- The pocess iscalled chemotaxis { bacteria signal each other and form groups}

6.MESOSOMES:

- These are invaginations of plasma membrane as vesicles tubes and lamellae they are present in both prokaryotic and eukaryotic cells they may be attached to the bacterial chromosomes and help in chromosomal replicaton or they are located next to the septa or cell wall and helpsin cell wall formation, they provide large surface area for greater metabolic activity.

7.CYTOPLASMIC MATRIX:

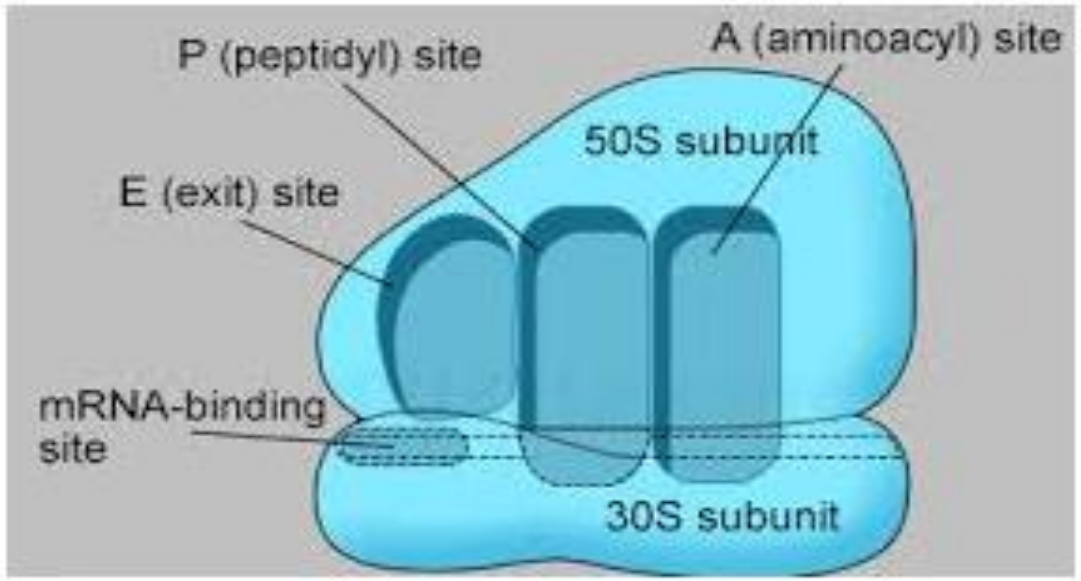
- They dont have membranebound organells.
- In prokaryotic cells it is a substance between nucleoid and plasma membrane.
- The matrix consistsof 70% of water packed with ribosomes and they are highly orgaanised.

- Same specific proteins are present in the cell pole and at the side the cell divides the plasma membrane along the cytoplasm and their organells are called protoplasm.

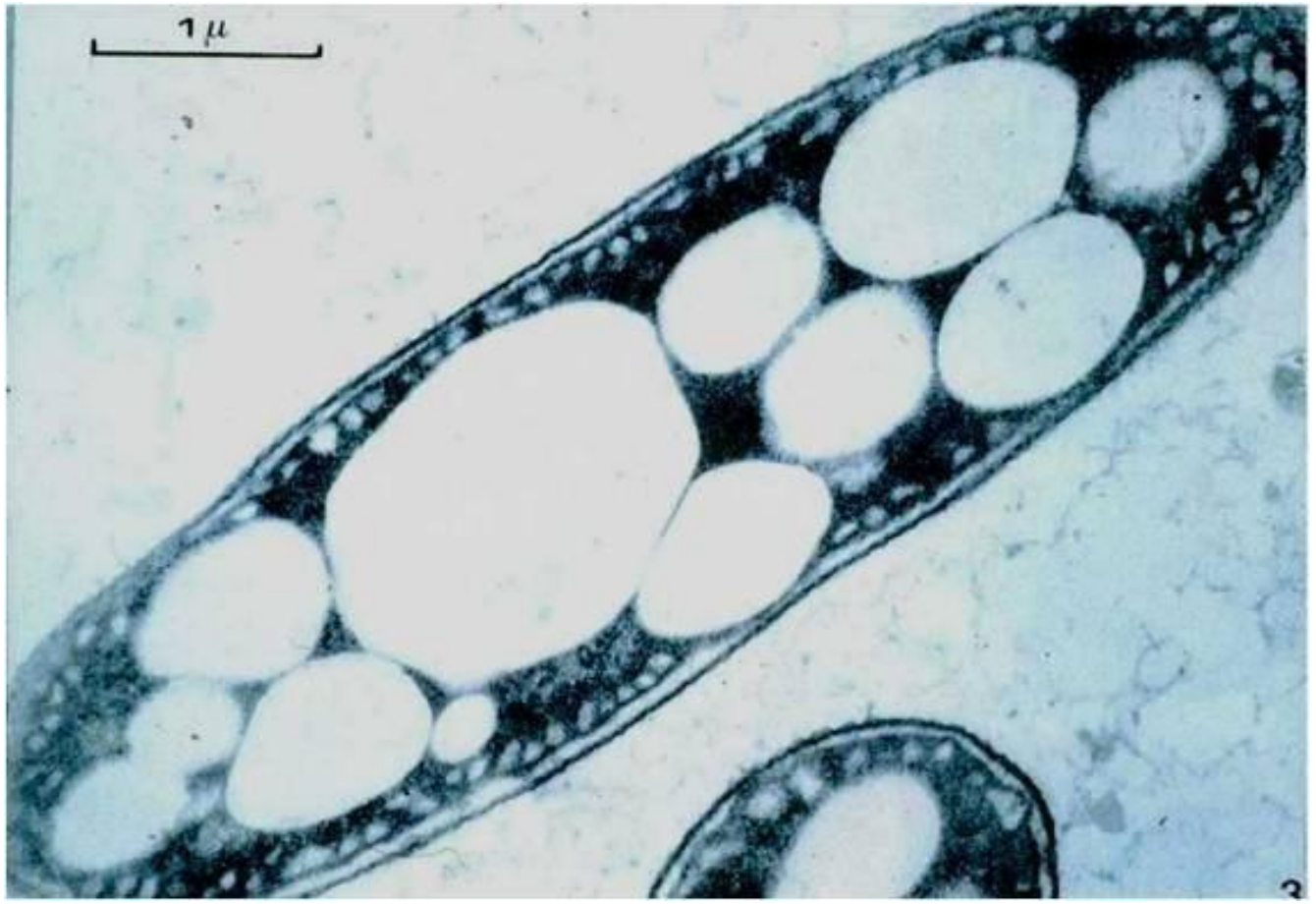
8.**RIBOSOMES:**

- Ribosomes are tightly packed in the cytoplasmic matrix or otherwise loosely attached to the plasma membrane.
- They are made up ofprotein and ribonucleic acid (RNA)
- It is the site of protein synthesis
- Based on placement(location) they are reffered as cytoplasmic matrix ribosomes or plasma membrane ribosomes.
- In cytoplasm matrix synthesize and keep the ribosomes inside cell, where as plasma membrane synthesize ribosomes and transport outside the cell.
- The polypeptides formed by ribosomes are immediately folded after the synthesis or else they will be folded after the completion of protein synthesis.

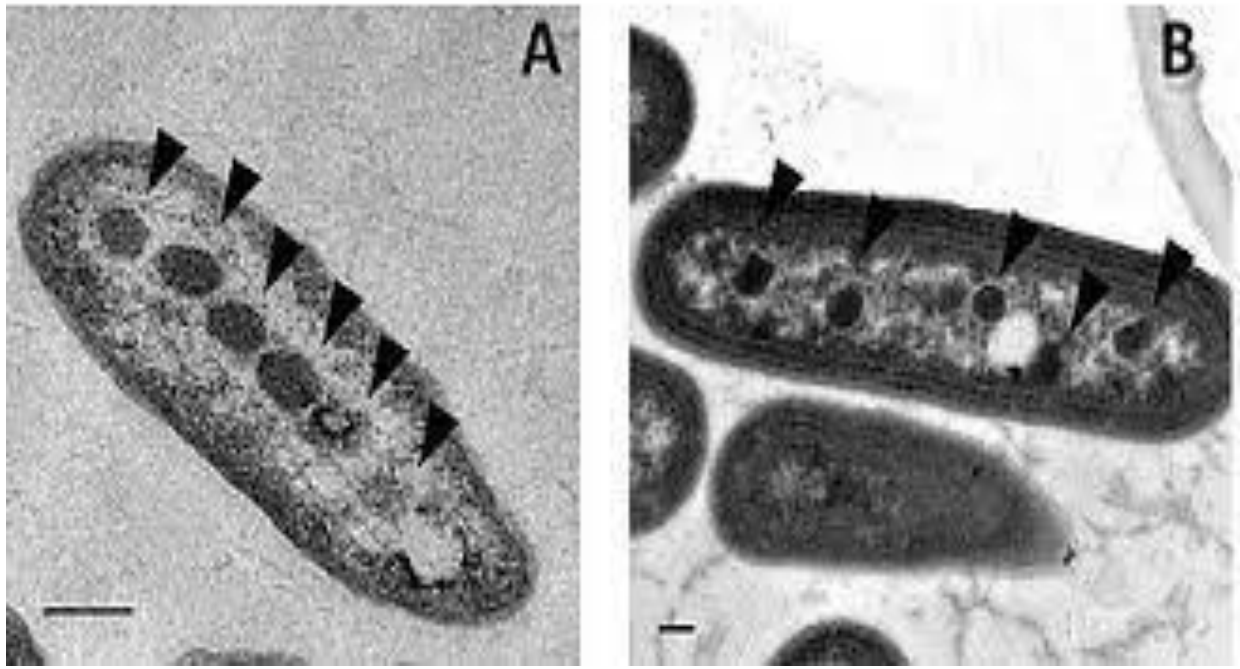
- The shape of protein is determined by its amino acid sequence.
- The prokaryotic ribosomes are smaller than eukaryotic ribosomes.
- The prokaryotic ribosomes has 2 subunits 50S larger subunit and 30S smaller subunit together they are 70S ribosome.
- These are 14-15 nm in diameter and 20nm in length. The “S” indicates sved berg unit which is the unit of sedimentation coefficient.
- SC- it is the measure of sedimentation velocity the faster a particle travels greater is the sved berg unit or S.C is the function of molecular weight shae and volume of the particle.



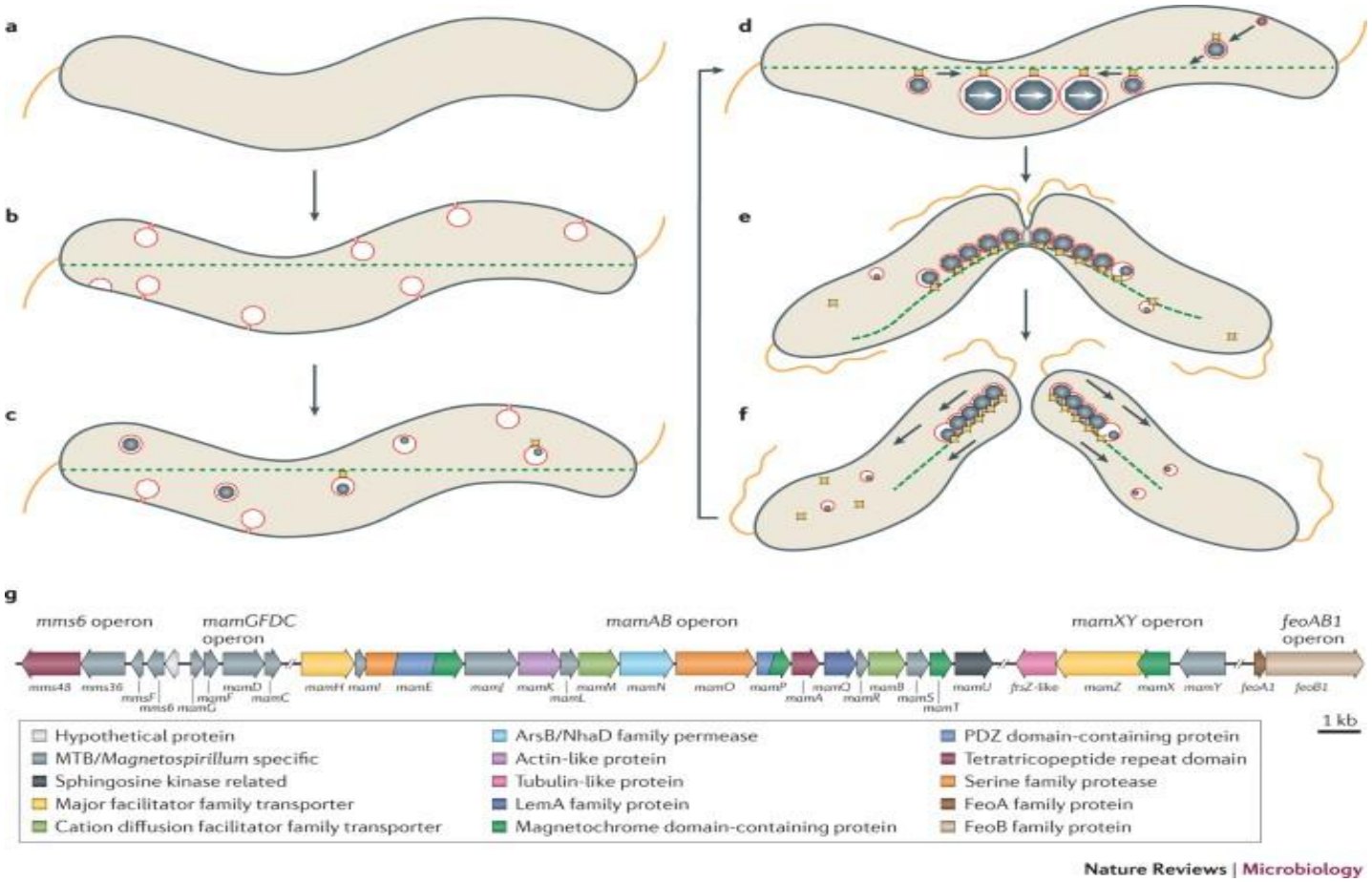
Gas vacuoles:



Carboxysomes

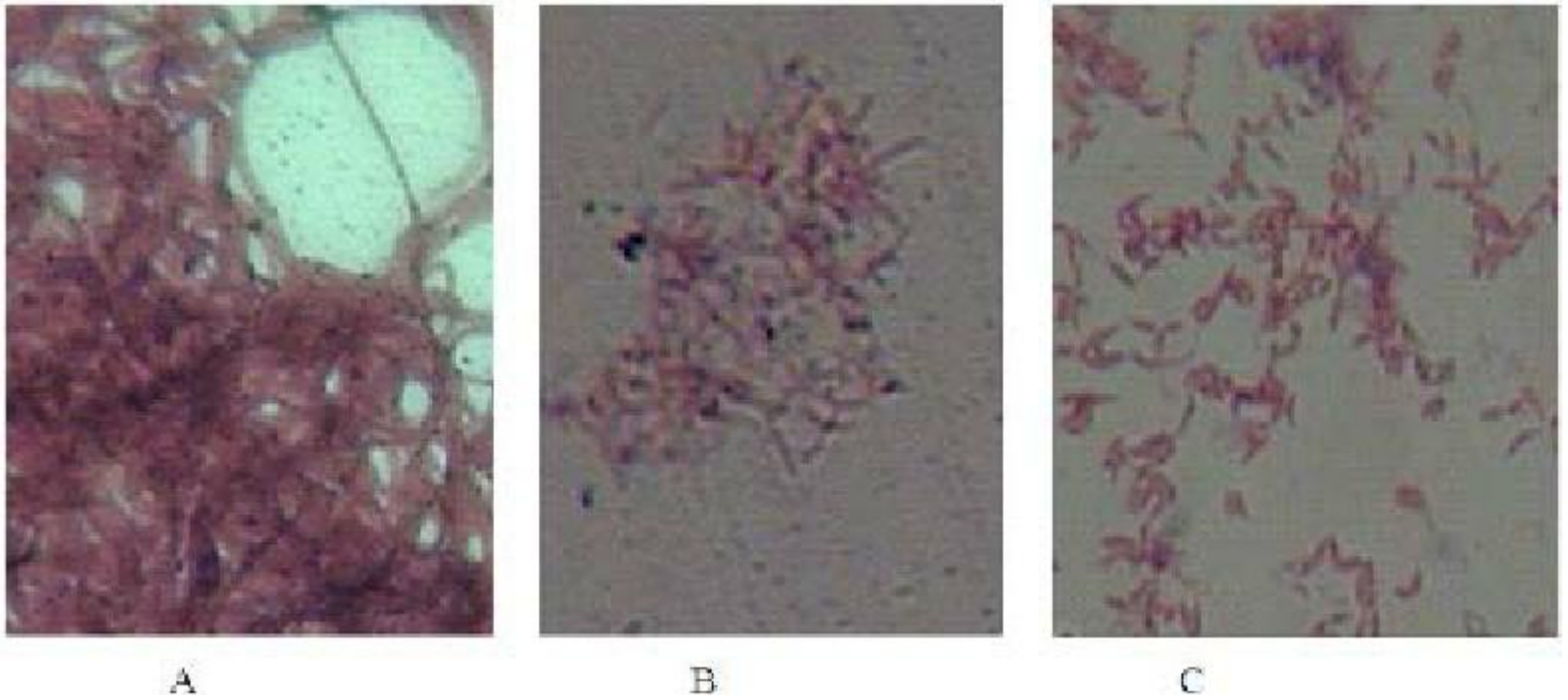


Magnetosomes:



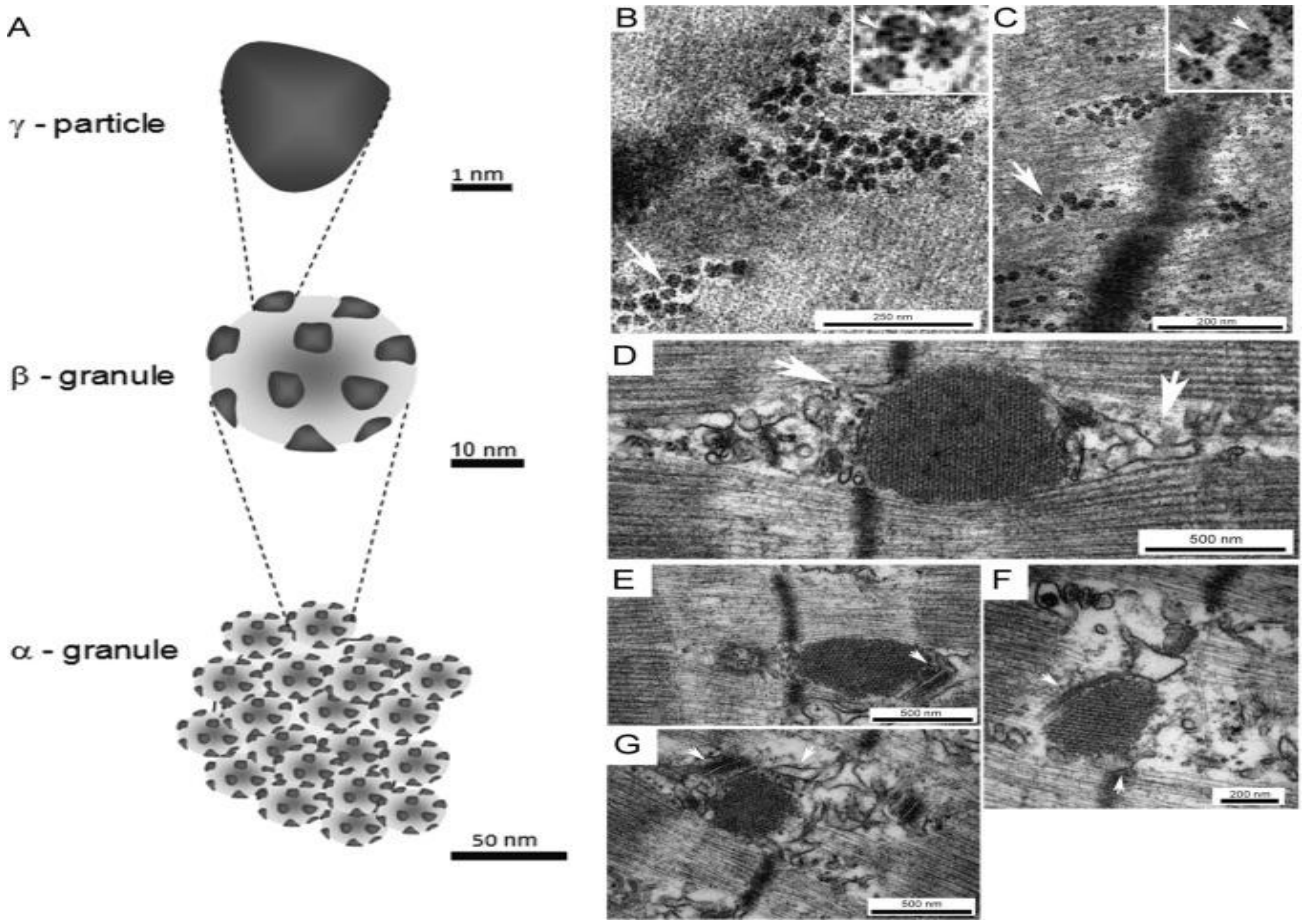
Inclusion bodies-

a)PHB granules:

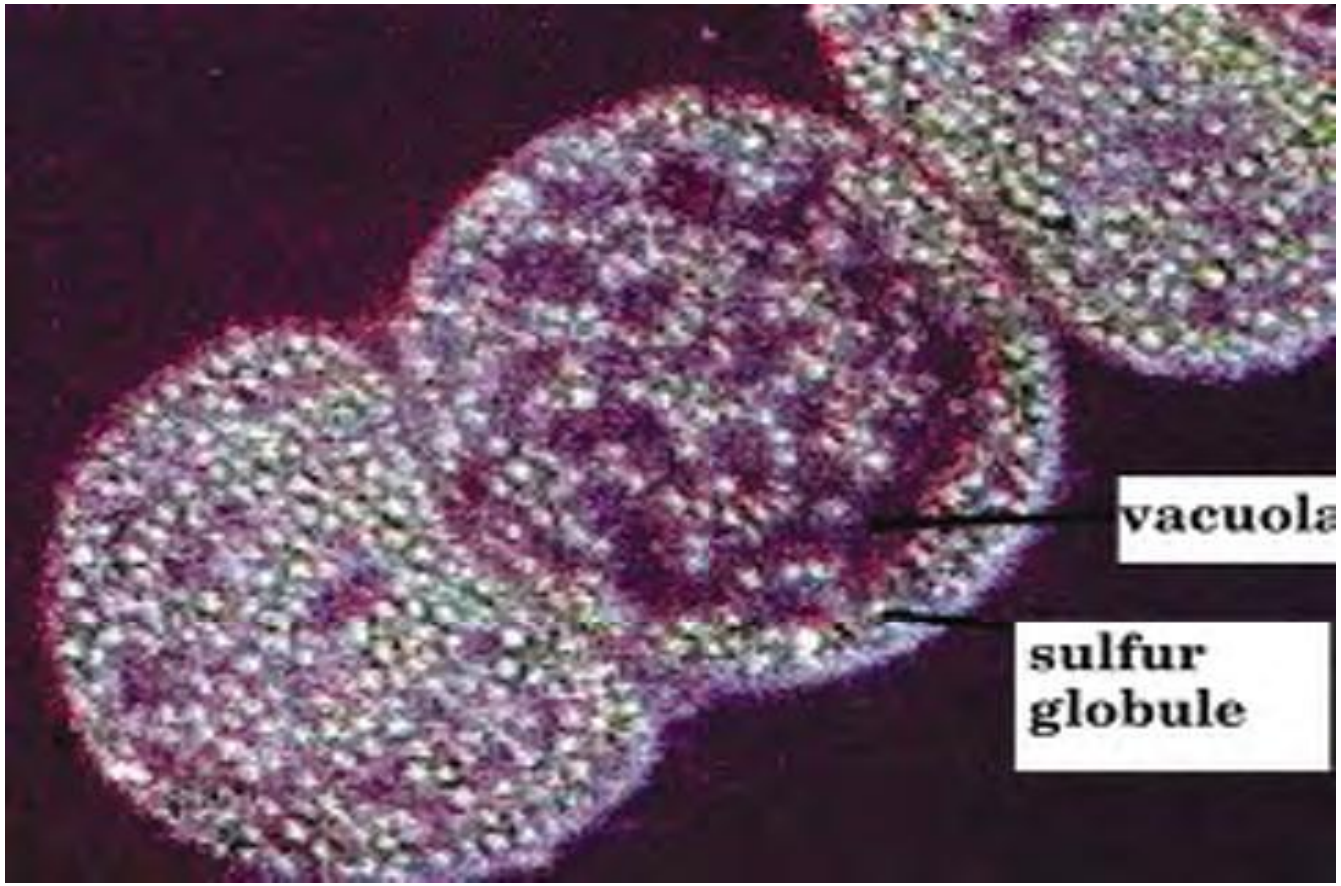


A B C

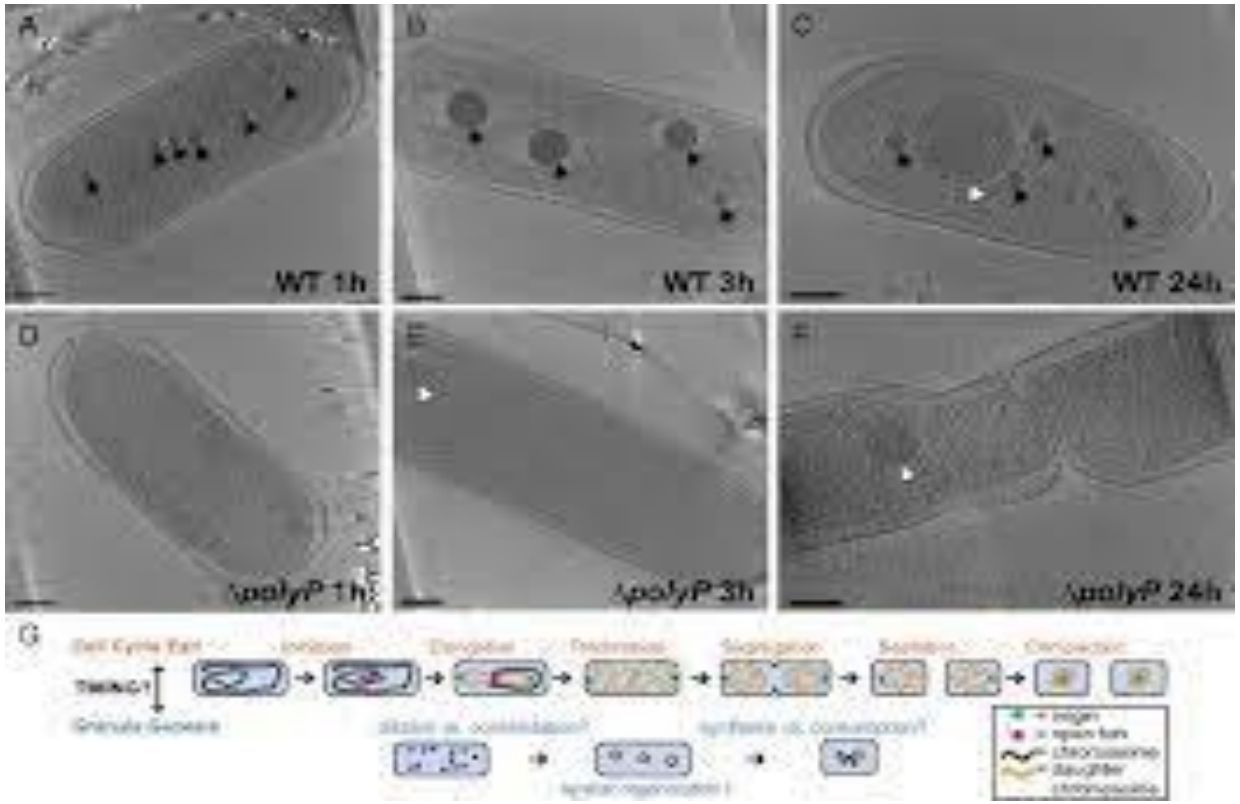
b) Glycogen granules



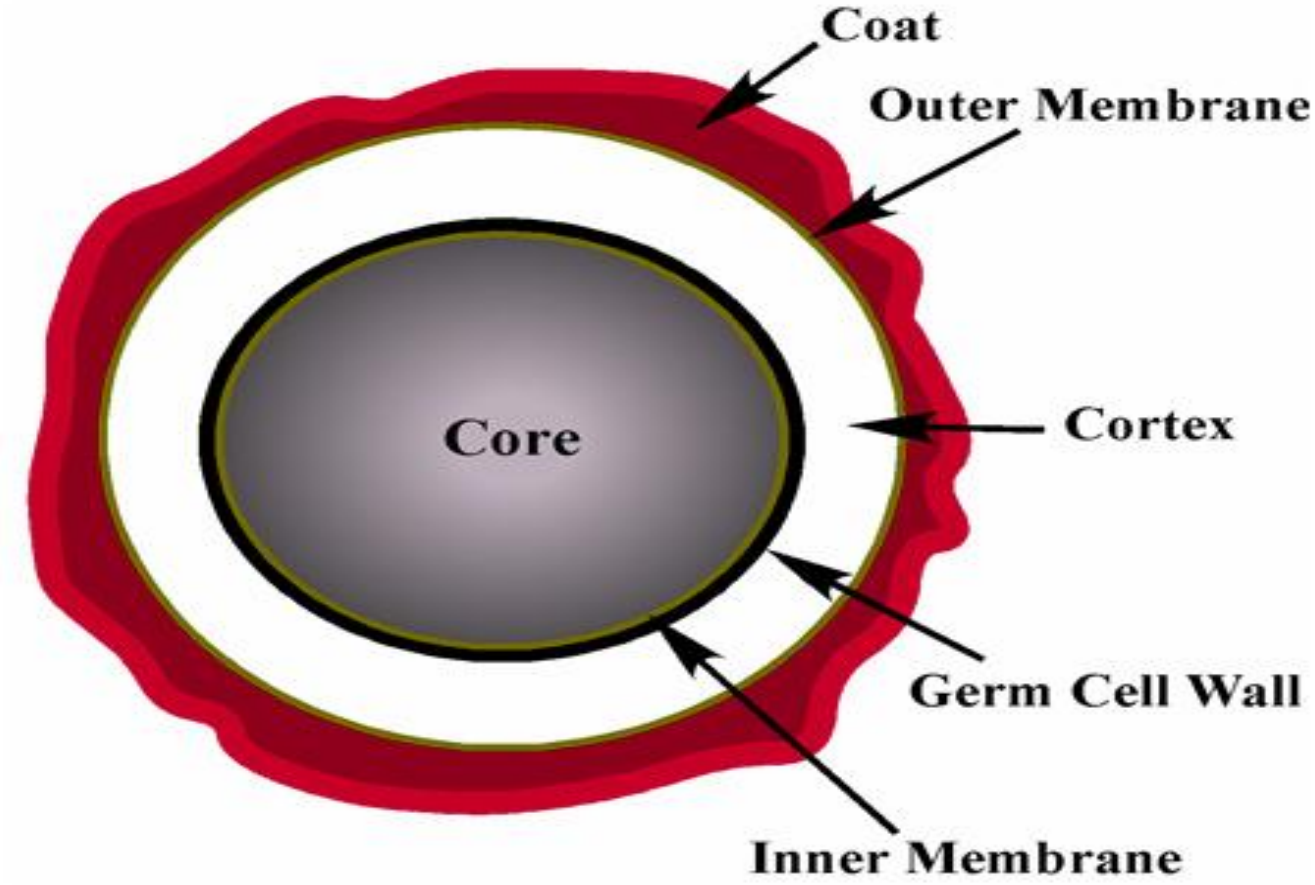
c) Sulphur granules



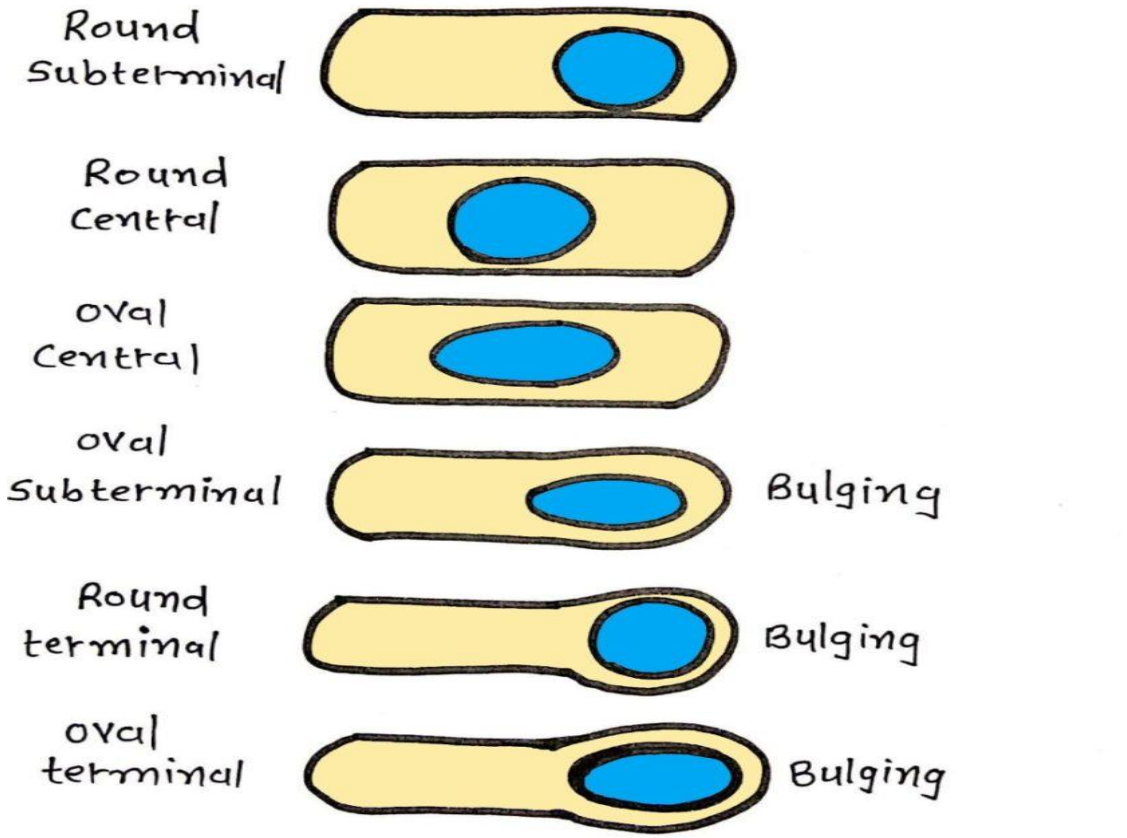
Polyhosphate granules:



Endospore:

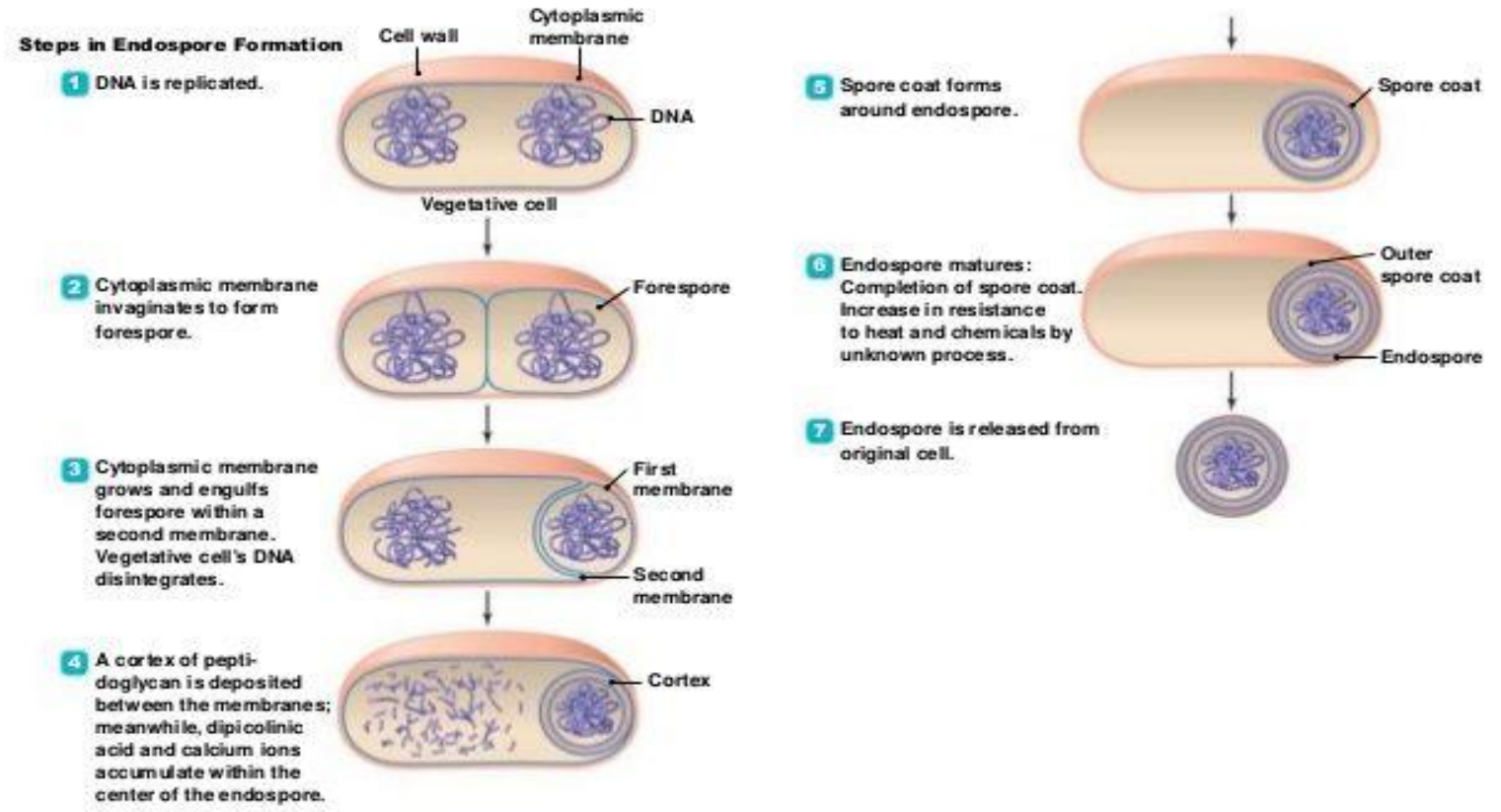

Coat
Outer Membrane
Core
Cortex
Germ Cell Wall
Inner Membrane

Types:


Round
Subterminal
Round
Central
oval
Central
oval
Subterminal
Bulging
Round
terminal
Bulging
oval
terminal
Bulging
SHAPE & POSITION OF BACTERIAL SPORES

Formation:

Figure 3.24 The formation of an endospore.



9.NUCLEOID:

- Nucleoid is unbounded by membrane. The chromosomes are present in irregular shaped region called nucleoid or nucleoid body or chromatin body or nuclear region.
- They possess single linear DNA or single circled double stranded DNA.
- Generally all the prokaryotic organisms contain 1 chromosome but vibrio cholerae have 2 chromosomes.
- The nucleoid can be observed under microscope using fuelgen staining.
- These nucleoids are made up of 60% DNA, 30%RNA and 10%protein.
- In addition to the nucleiod an extrachromosomal DNA called “Plasmids” are present in bacteria.
- It is a circular double stranded DNA, they exist and replicate independently and also they can integrate with chromosomal DNA and can be inherited to their progeny.
- Plasmids are not necessary for host growth but they carry genes responsible for drug resistance.

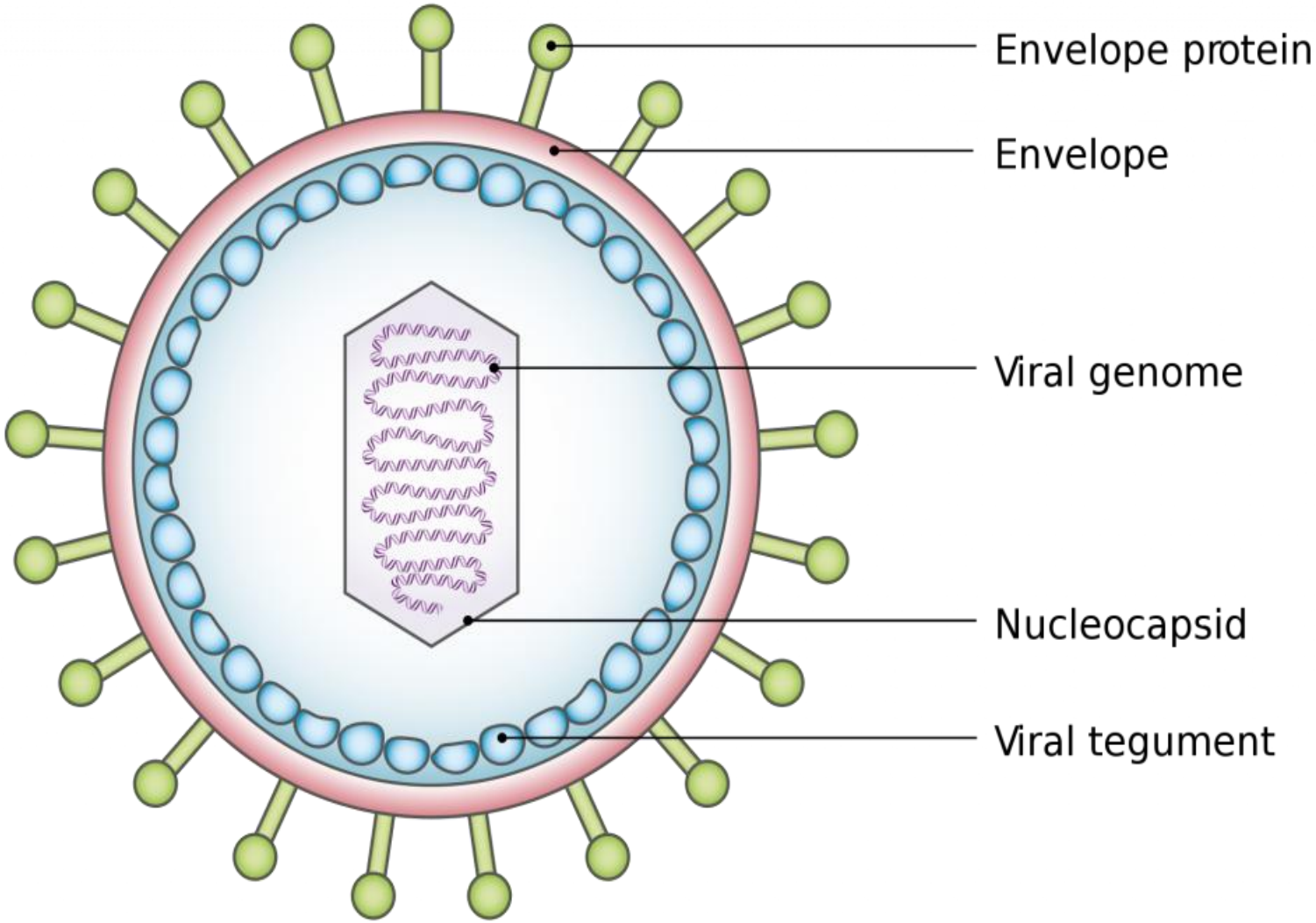
TOPIC -2
GENERAL CHARACTERISTICS OF VIRUS AND CLASSIFICATION:

Definition:

Viruses can be defined as noncellular, submicroscopic,obligatory intracellular parasites having protein coat around the nucleic acid (DNA/RNA) and capable of self-replicating within the host cells.

- Viruses are unique class of infectious agents truely distinctive by their simple organization and mechanismof replication.
- The study of virus is called as virology and specialists in this field are called virologists.

STRUCTURE:


Envelope protein
Envelope
Viral genome
Nucleocapsid
Viral tegument

- Viruses are:
- Unique class of infectious agents, simple in structure and replication.
- Viruses occur in 2 states
- a) Extracellular- a virus is minute particle containing nucleic acid surrounded by protein coat and occasionally depending on specific viruses they contain other macro-molecular components also. In this extracellular form the virus particle is known as virion.
- It is metabolically inert and does not carry out respiratory or biosynthetic activity.
- The virion is the structure by whichthe virus genome is carriedfm the cell in which it has been produced into other cell where the virus nucleic acid can be introduced.
- b) Intracellular –The virus once it enters inside a new cell, the intracellular stage is initiated.
- In this stage virus replication occurs, new copies of genom are produced and the components that make up the virus coat are synthesized.
- Since virus has nucleic acid and protein called nucleoprotein.
- Protein coat is called as capsid which encloses the genome.
- Capsid has capsomeres, these are individual protein units.

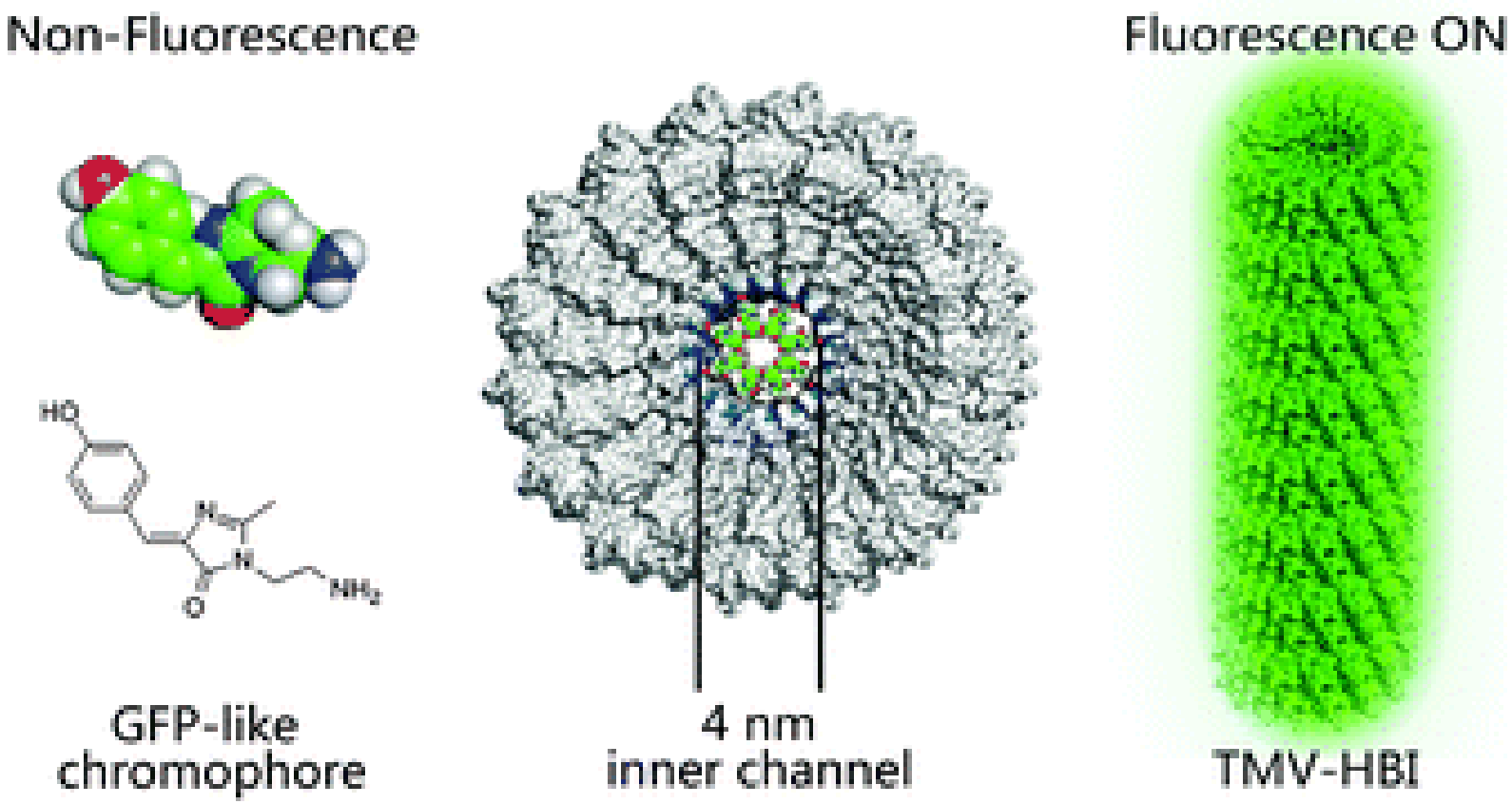
Characteristics:

- All the viruses are obligate parasites, Acellular.. they dont posses highly developed structures.
- They multiply only in a living host cell and remain inert ouside the host cell.
- They are ultra microscopic and can only be viewed with electron microscope(the smallest known virus is merely 0.002um in diameter,, while the largest ones are typically about 0.8um in diameter)
- Viruses are actually nucleoproteins. The viral genome may be either DNA or RNA.
- Viruses are usually minute that they can easily pass through a filter, which can hold back even the smallest bacteria.
- Viruses don't have metabolic energy system of thier own hence uses host cells and antibiotics have no effect on them.
- Viruses don't response to external stimuli.
- Maintain genetic continuity.
- Viruses generally infect bacteria,fungi,plants,animals, and humans
- Size of a virus ranges from 10 to 200nm
- Latest virus is equal to small bacteria.

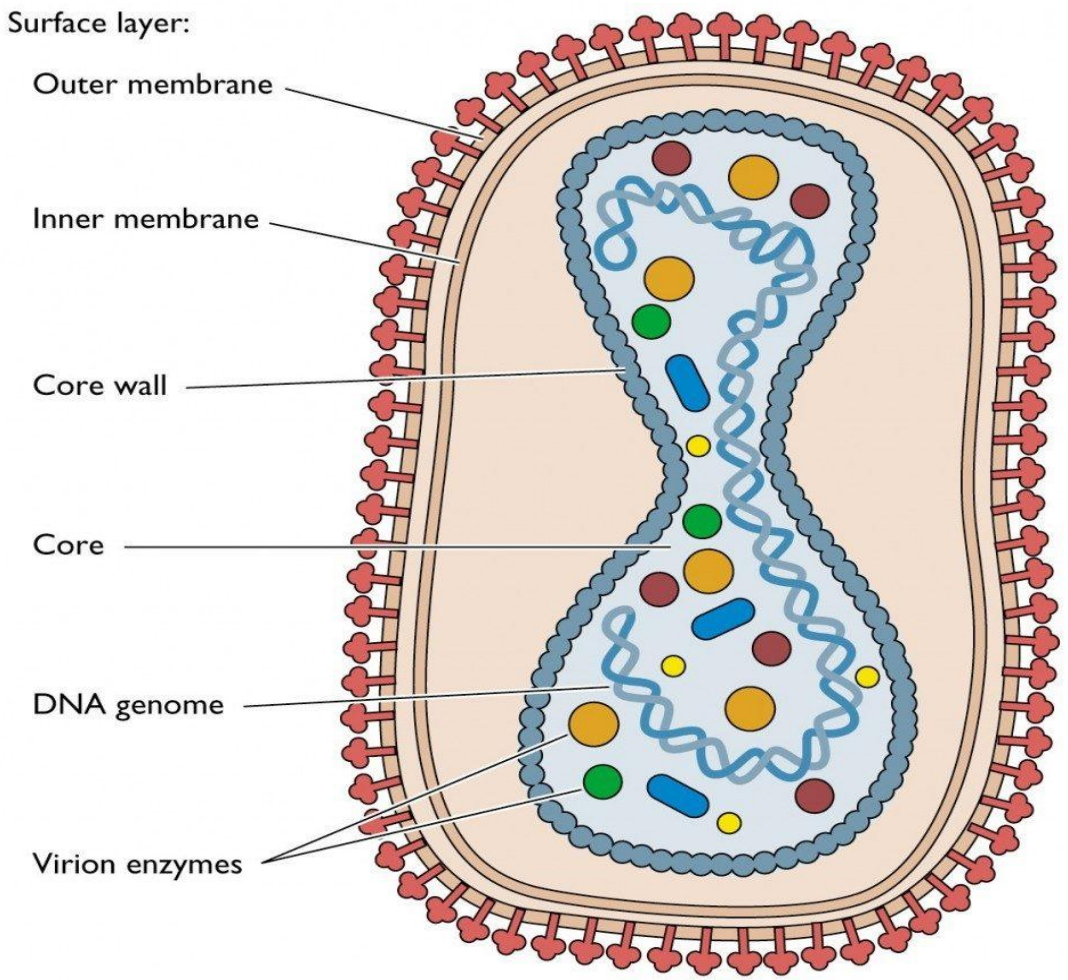
Shapes of viruses:

SHAPE	VIRUS EXAMPLE
Rod shaped or elongated	TMV
Rectangular	Pox viruses
Polyhedral	Adenoviruses
Spheroidal	Polioviruses
Tadpole shape	T-even phages
Bullet shape	Rhabdoviruses

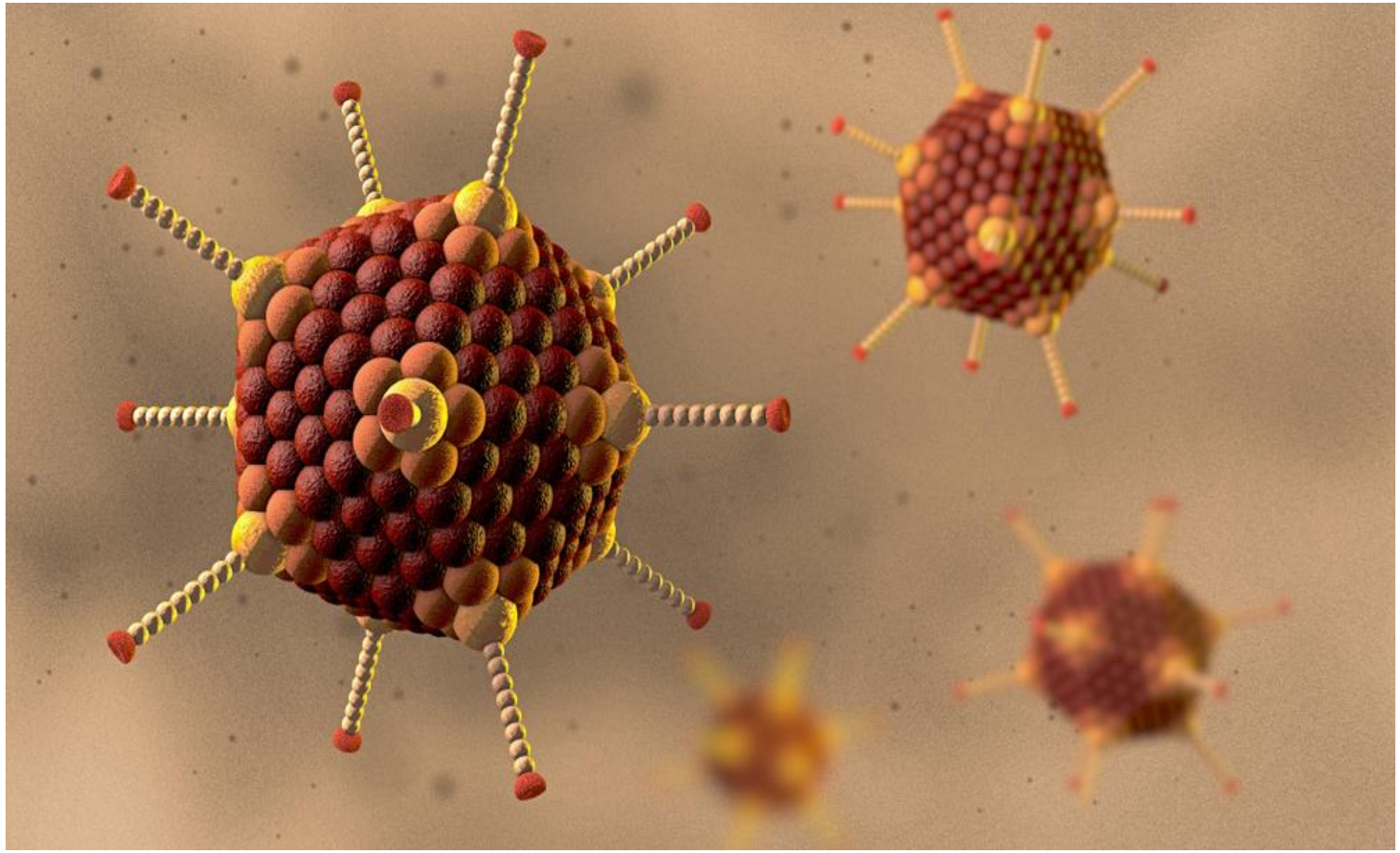
Rod shaped or elongated: TMV:



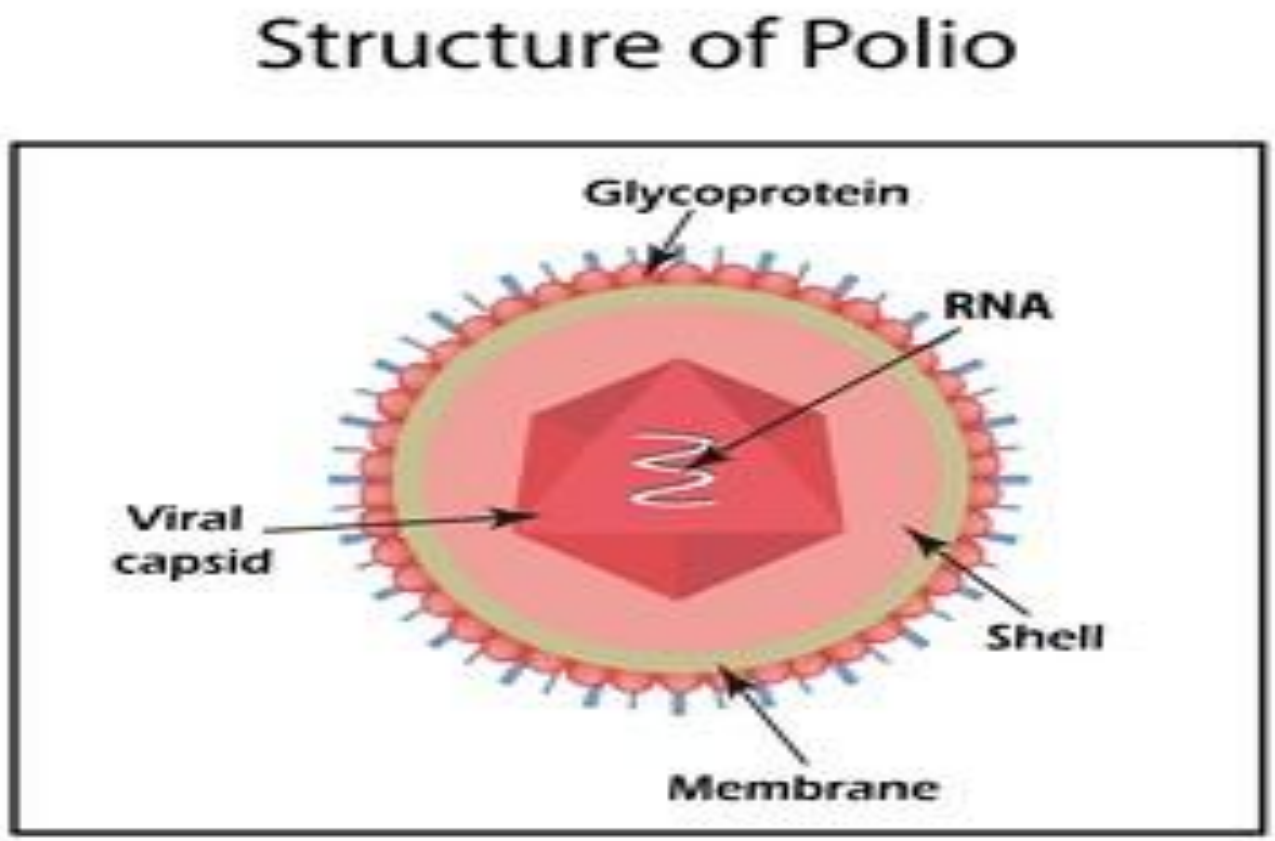
Rectangular: Pox viruses



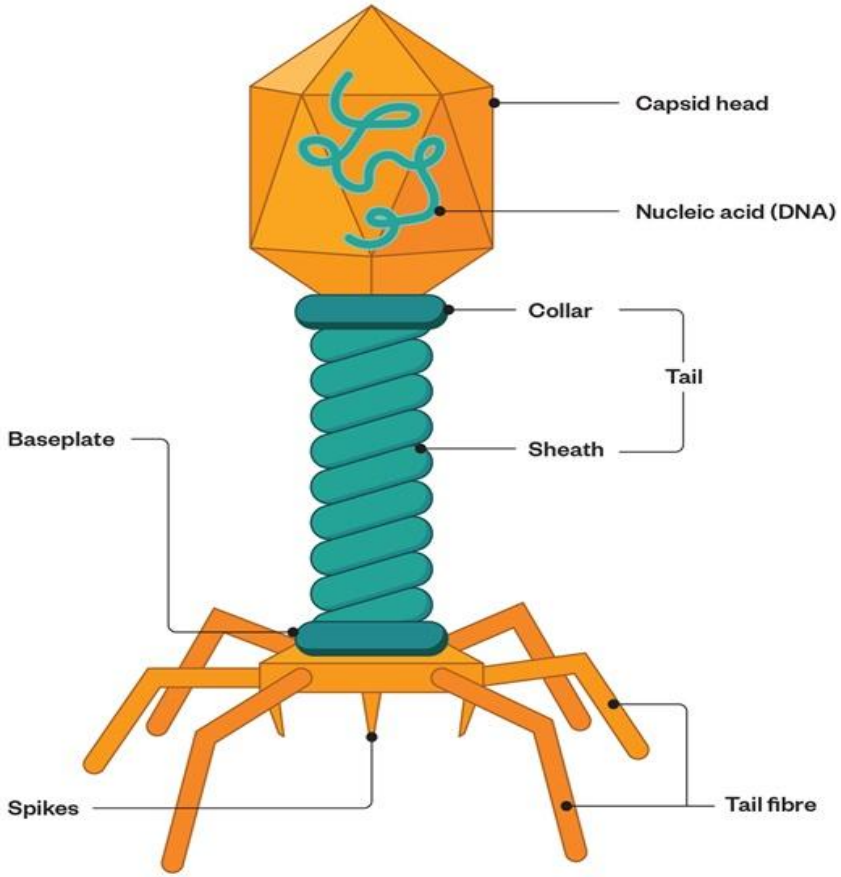
Polyhedral: Adenoviruses



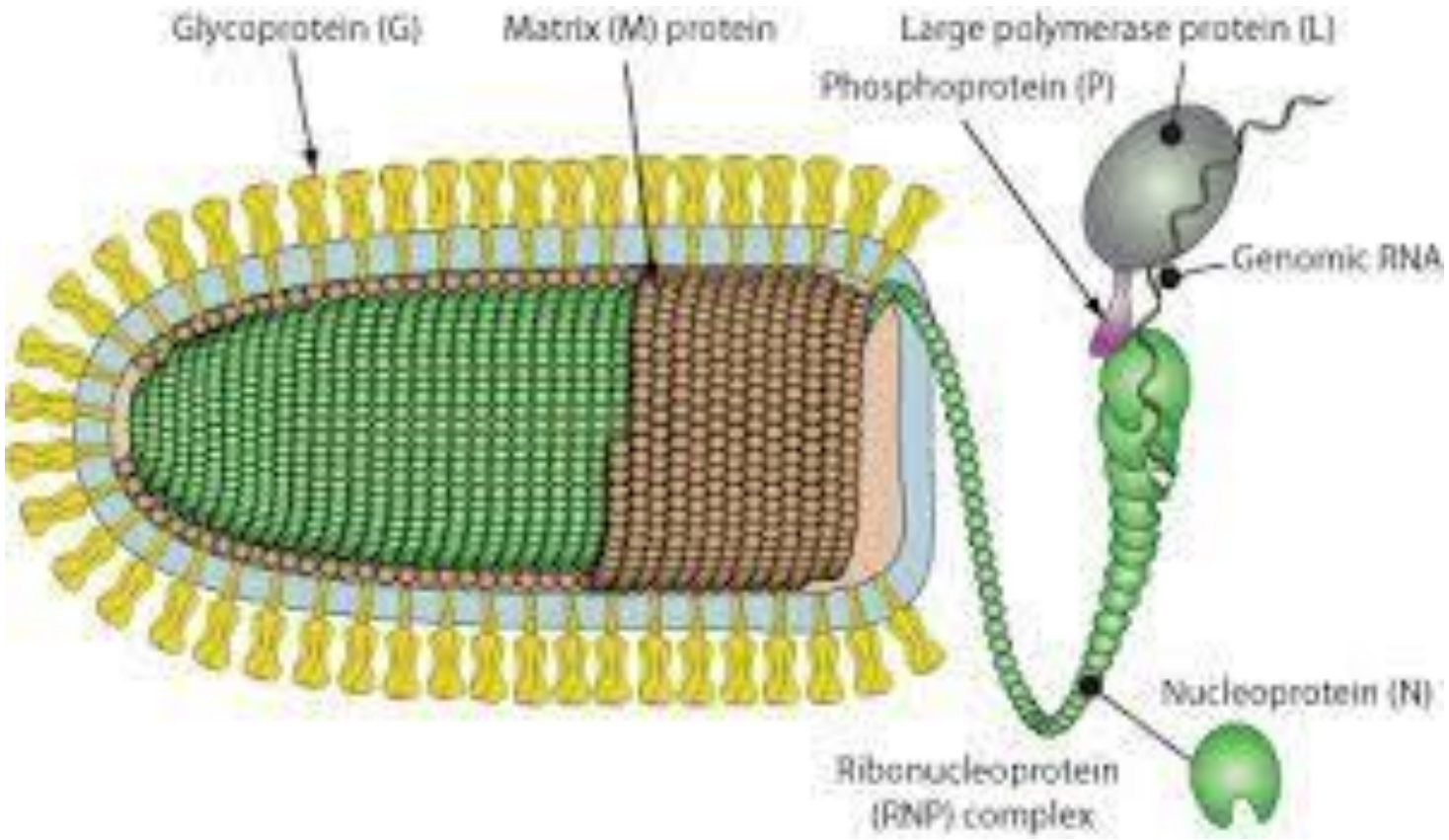
Spheroidal : Polioviruses



Tadpole shape: T-even phages



Bullet shape : Rhabdoviruses



SIZE OF VIRUSES:

- 10-200nm
- Polio myelitis, yellow fever, foot and mouth disease causing viruses range upto 25nm.
- Small pox 250nm in diameter.
- Smallest viruses (mouth and foot)- 10nm
- Herpes simplex virus- 100-150nm
- Mumps virus-150-200nm
- Influenza -80-120nm
- Adeno- 80-90nm

Classification of virus:

1. Based on infection caused on different organ.

2.Based on tropical- specific area

- Dermatotropic viruses – The viruses which attack skin region e.g small pic, chicken pox.
- Neurotropic viruses –The viruses whose target is CNS(central nervous system) e.g polio virus
- Pneumotropic viruses –The viruses that attack respiratory system. e.g Influenza, flu, cold.
- Viscerotropic viruses –The viruses that attack vital organs(visceral organs) – Hepatitis (lungs)

3.Nature of genome : DNA/ RNA

4.Segments of genome: Based on how many fragments that the genome is divided into. e.g Adeno virus, HIV, Influenza.

5.Depending on amount of G+ C content.

- 6. Morphological features
- □ size , shape
- □ symmetry(Icosahedral, Helical,complex)
- □ account of no of capsomeres
- □ evenveloped or spikes
- □ 7.Physico chemical properties- molecular weight, sensitivity.
- □ 8.Presence of antigens (surface antigens, reverse transcriptase enzyme,
- Neuraminidase.
- □ 9.specificity of host e.g human or animals (rabies)

10. Organ specificity

- 11.site of multiplication (nucleus or cytoplasm)
- 12.Transcriptional patterns: Either the virus uses its own gene or hosts genome)
- 13.Mode of transmission: Air, water, water droplets.

Classification:

Baltimore classified viruses Into 6 major classes

Based on

- 1. Nature of genome
- 2. Mode of replication
- 3.Gene expression.

4. Based on revised scheme on fundamental importance of mRNA in the replication cycle.

I) DNA viruses – 1. ss DNA

- 2. ds DNA

II) RNA viruses – 1. ss RNA

- 2.ds RNA

III)RNA – DNA viruses

- 1.ssRNA(retro viruses)
- 2. ds RNA (hapadnaviruses)

1. **Ds DNA viruses:**

- There is no deignation of + and - strands in the DNA.
- The transcription is similar to that of host cells.

 Animal viruses : Papovavirus

  Adenovirus

  Herpes virus

  Pox viruses

 Plant viruses : Cauliflower mosaic viruses

  Dahlia mosaic viruses

 Bacterial viruses : E.coli bacterophages T1, T2

 Cyanoviruses : N1 pages

Ss DNA viruses :

In this the single stranded must be converted into double stand before synthesizing mRNA.

- **Animal viruses**: Parvoviruses
- **Plant viruses**: chlorosis striate mosaic viruses
- **Bacterial viruses**: E.coli bacterophages 174, S 13

Ds RNA viruses :

1) The genome is usually segmented.

Animal viruses: Reoviruses ,

Rota viruses

- Plant viruses: Rice dwarf viruses
- Bacterial viruses: bacteriophage 6

- **<u>SS RNA viruses:</u>**

Animal viruses: picornaviruses (polio virus)

- togaviruses
- paramyxovirus
- orthromyxovirus (influenza viruses)
- Rabdo viruses

Plant viruses: TMV

- potato dwarf viruses

Bacterial : Bacteriophage MS2

TOPIC -3 TMV (TOBACCO MOSIACVIRUS)

- Structure of Tobacco Mosaic Virus:
- Shape: TMV possesses a rod-like shape, where the RNA genome shows a helical symmetry and the capsid protein subunits show radial symmetry.

- Size: TMV comprises a length of 300 nm and a diameter of 18 nm with a molecular weight of 39X106 Daltons.

CLASSIFICATION:

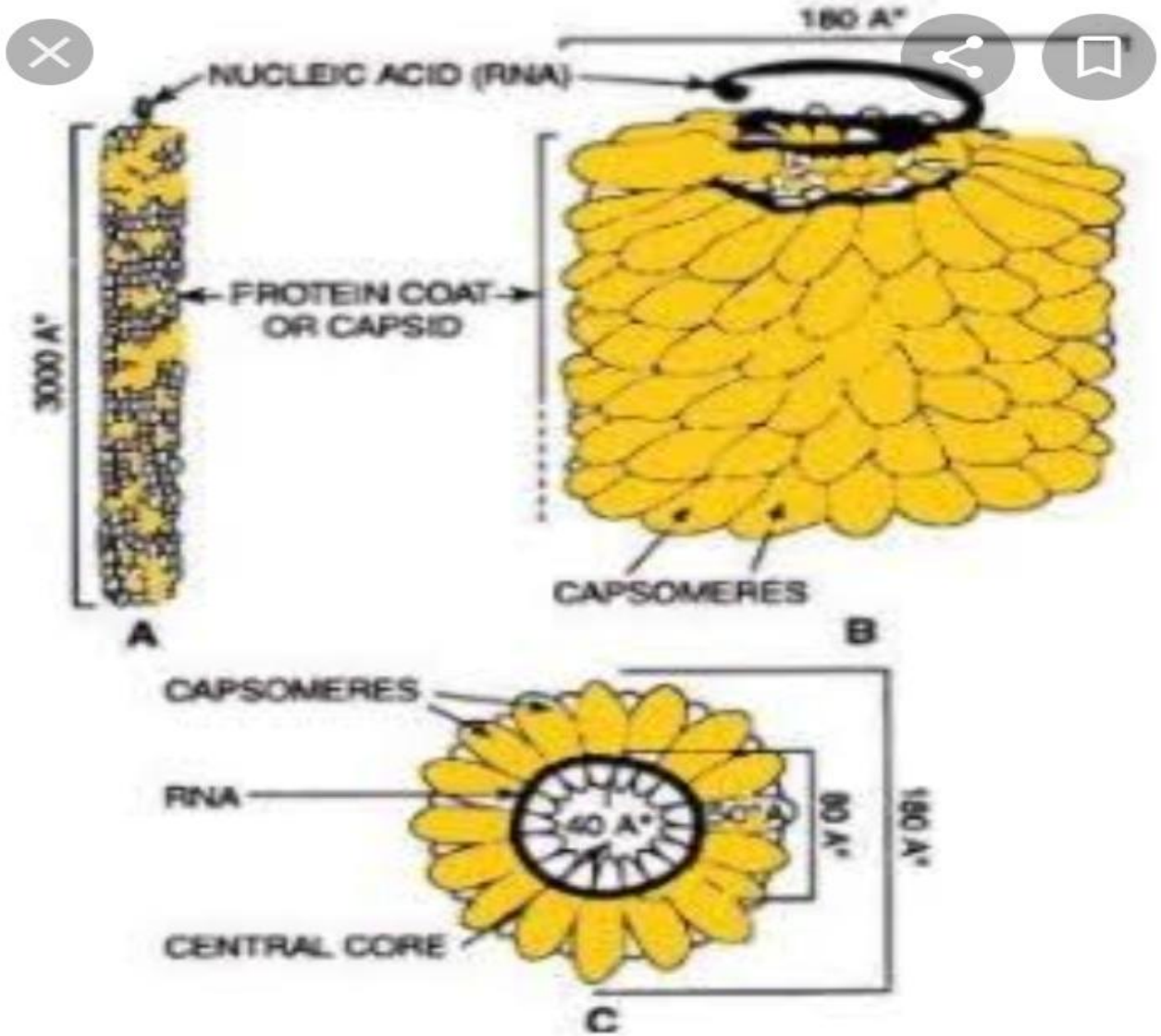
- Group: Tobamovirus
- Order : unassigned
- Family : Vigraniridae
- Genus : Tobacco virus
- Species : TMV

History:

- In 17^{th} and 18^{th} century there was huge loss of tobacco crop.
- Sap (leaf juice) of tobacco is taken and heated at 55c.
- They observed that infection is still not gone.
- They passed this sap through membrane filter and concluded that
- Beijernick – Contagium Vivum fluidic.
- Iwanosky – filterable agents
- W.M Stanley- isolated this virus in crystalline form.(noble prize)
- Fredrick C. Bowden and Norman W.Pirie separated the TMV particles into proteins and nucleic acid.

Structure :

- TMV is rod shaped helical virus measuring about 300nm long and 15-18 nm in diameter and weighing 39×106 Dalton.
- Genetic material is ss RNA and it is 6400bp long.
- Protein coat is present around the genome.
- Protein coat / capsid is having 2130 capsomeres.
- Each capsomeres is having 158 amino acids and it is about 17,500 Dalton in weight
- RNA is coiled into a helix and has molecular weight of 2.06× 106 Dalton.


180 A°
NUCLEIC ACID (RNA)
PROTEIN COAT
OR CAPSID
3000 A°
CAPSOMERES
A
B
CAPSOMERES
RNA
CENTRAL CORE
40 A°
80 A°
180 A°
C

- **Capsid:**
- The virion contains 2130 identical protein subunits, which also refers to as “Capsomers“.
- Each protein subunit comprises a single polypeptide chain of 158 amino acid residues with a molecular weight of 17,500 Daltons.
- The number of protein subunits in three turns of RNA is 49, or we can say a single protein subunit is linked with three nucleotides of the RNA genome.

- **RNA:**
- TMV possesses ssRNA genome with a hollow central cavity of diameter (4 nm).
- The RNA molecule is spirally twisted to form a helix, where one turn of helix contains 49 nucleotides.
- Generally, the virus RNA possesses 6500 nucleotides.
- It is protected from the cellular enzymatic action by the surrounded capsid.

Replication:

1. Adsorption:

Mechanically transmitted- Scratch , injury

Hence the virus wants damaged cuticle and cellwall of the host to be infected.

Penetration:

Virion enter into cytoplasm through damaged sites in the cell wall and plasma membrane.

Uncoating:

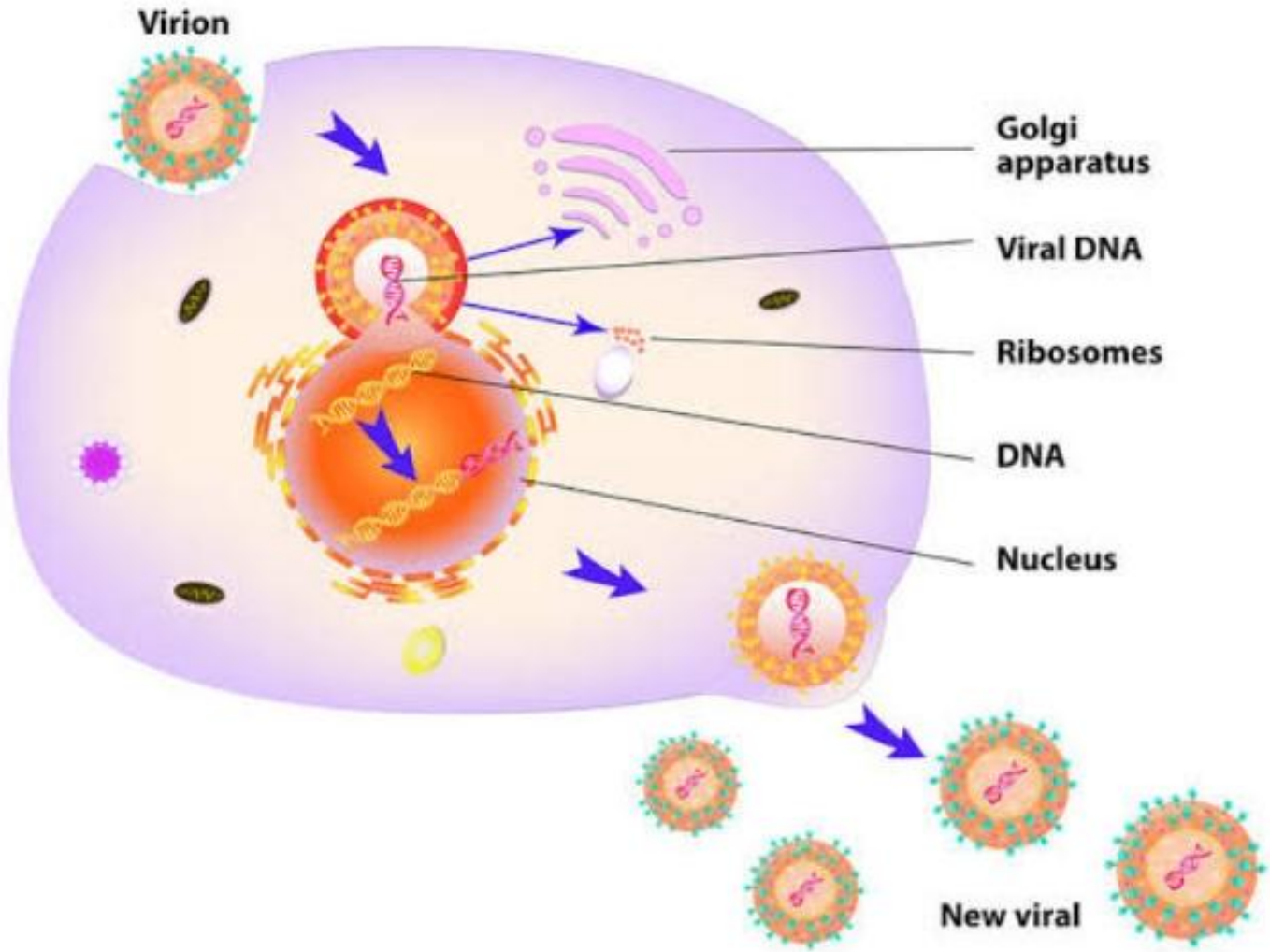
- Removes the protein coat and release the RNA into the cytoplasm.
- This process is called uncoating

Translation and transcription of genome:

- Inside the nucleus the viral RNA first induces the formation of specific enzymes called as RNA polymerase.
- In the presence of this enzyme the ss RNA synthesizes an additional RNA strand called ‘replicative RNA’.
- This RNA strand is complementry to the viral genome and serves as a template for producing new RNA single strand which are copies of parental viral RNA.
- The new viral RNA are released from nucleus into cytoplasm

Assembly:

- It is the process of assembly of coat proteins to form capsid and also association of RNA with capsid to form complete virion.


Virion
Golgi apparatus
Viral DNA
Ribosomes
DNA
Nucleus
New viral

TOPIC -4: HIV (Human immunodeficiency virus)

- HIV is a retro virus (a virus that belong to Retroviridae family) and causes aquired immunodeficiency syndrome (AIDS) in humans.
- It is divided into two major types HIV 1 and HIV 2.
- The HIV1 is supposed to be the american strain, i.e it has typical characteristics found in the blood smaples of patients from USA, Europe and HIV2 has been discovered in eastern africa.
- Currently 90% of total global AIDS cses are dueto HIV1.
- HIV is characterised by severe reduction in the CD4+ T cells.
- It means that the infected patient is weak and vulnerable to many diseases.

HISTORY:

- The human immunodeficiency viruses 1 and 2 (HIV-1, HIV-2) originated from the simian immunodeficiency viruses (SIVs) of primates.
- Thus, HIV-1 and HIV-2 each had a zoonotic origin but now spread directly from human to human.
- HIV-1 was first isolated in 1983 and HIV-2in 1986 and they represent two different epidemics.

HIV structure:

1)The human immunodeficiency viruses are approximately 100 nm in diameter

2) It has a lipid envelope, on which are embedded the glycoprotein gp41 to which the surface glycoprotein gp120 is attached

3)These two viral proteins are responsible for attachment to the host cell and are encoded by the env gene of the viral RNA genome.

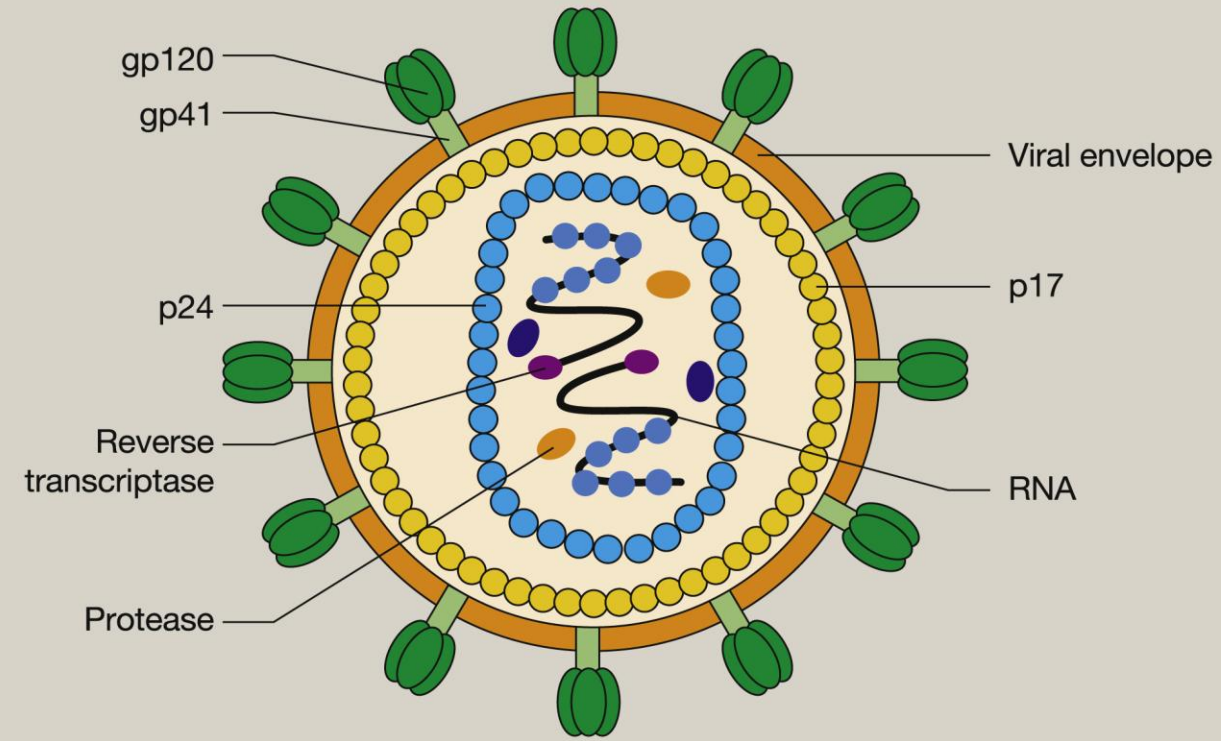
4)Beneath the envelope, is the matrix protein , the core proteins and the nucleocapsid.

5) Within the viral core, lies 2 copies of the positive-sense, viral RNA genome

6) These together with the protease, integrase and reverse transcriptase enzymes.

7) These three enzymes are encoded by the viral pol gene

Human immunodeficiency virus – structure



- Antibodies to HIV typically appear 4–6 weeks after infection, but this may take as long as 3 months
- The p24 antigen can usually be detected in a blood sample from 2–4 weeks after infection
- The p24 antigen becomes rapidly undetectable once antibodies to HIV start to develop

HIV replication:

- The main attachment receptor for HIV is the CD4 molecule that is present on the CD4 positive T (helper) lymphocyte, macrophages, and microglial cells.
- The viral gp120 binds initially to this CD4 molecule, which then triggers a conformational change in the host-cell envelope that allows binding of the co-receptor which is required for fusion between virus envelope and cell membrane.
- All retroviruses encode an reverse transcriptase enzyme that transcribes its viral RNA into double-stranded DNA (dsDNA), which is then integrated, via the action of the integrase enzyme into the host-cell genome .
- The viral integrated dsDNA or 'provirus' then acts as a template for viral genomic and messenger RNA transcription by the host cell's nucleic acid replicating machinery.
- Recombination between these two RNA strands during viral replication, coupled with the extremely error-prone action of the RT enzyme, give rise to the extreme genetic diversity of HIV.

- Integration of the linear provirus dsDNA into the genome of the host-cell establishes an infection that lasts for the lifespan of the cell, and all its progeny, which usually means life-long infection for the organism, in this case the human host.
- Like all retro viruses genome, HIV contains the 3 basic genes, the gag geenes, pol genes, env gene with other five genes of Q,R, tat, art/ trs and F.
- The gag encodes for- proteins of internal structure.
- The pol gene encodes- reverse transcriptsae.
- The envelope genes- envelopee glycoprotein.
- The function of tat and art/trs gene are knoen ande they are connected with rplication of virus.
- When the synthesis of core protein, envelpe protein and reverse transcriptase is over in the infected cell, packaging of on particles takes place.
- Later virions are released from the infected cells by budding process.

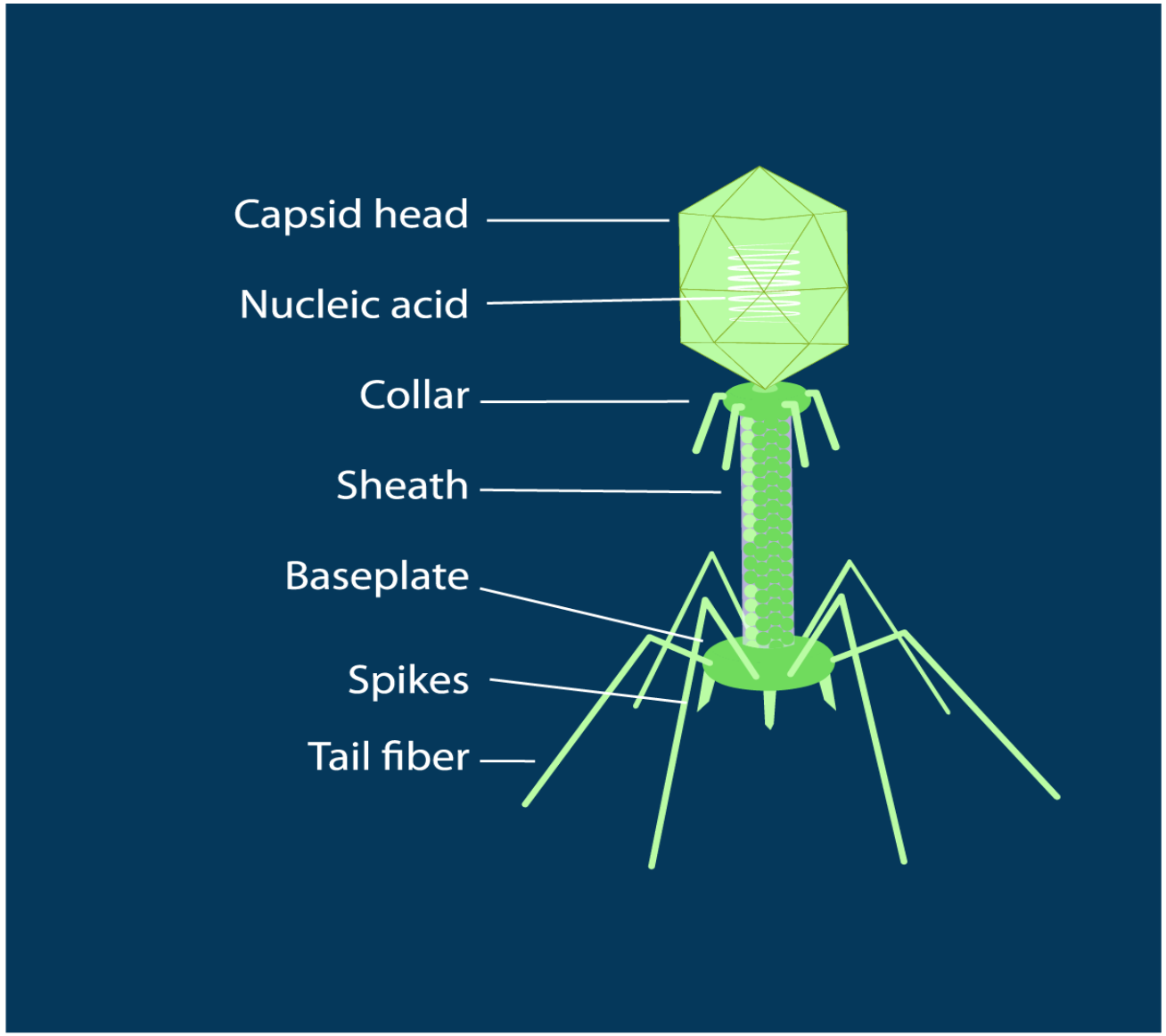
TOPIC -5:BACTERIOPHAGE (Lambda phage)

- Bacteriophages are viruses that parasitize bacteria.
- Bacteriophages were jointly discovered by Frederick Twort (1915) in England and by Felix d'Herelle (1917) at the Pasteur Institute in France.
- Felix d'Herelle coined the term “Bacteriophage”.
- Bacteriophage means to eat bacteria, and are called so because virulent bacteriophage can cause the compete lysis of a susceptible bacterial culture.
- They are commonly referred as “phage”
- Phages are obligate intracellular parasites that multiply inside bacteria by making use of some or all of the host biosynthetic machinery.
- They occur widely in nature and can readily be isolated from feces and sewage.
- There are at least 12 distinct groups of bacteriophages, which are very diverse structurally and genetically.

Morphology:

- Most phages range in size from 24-200 nm in length
- T4 is among the largest phages; it is approximately 200 nm long and 80-100 nm wide.
- All phages contain a head structure, which can vary in size and shape.
- Some are icosahedral (20 sides) others are filamentous.
- The head encloses nucleic acid and acts as the protective covering.
- Some phages have tails attached to the phage head.
- The tail is a hollow tube through which the nucleic acid passes during
- infection.

- T4 tail is surrounded by a contractile sheath, which contracts during infection of the bacterium.
- At the end of the tail, phages like T4 have a base plate and one or more tail fibers attached to it.
- The base plate and tail fibers are involved in the binding of the phage to the bacterial cell.
- Not all phages have base plates and tail fibers.


Capsid head
Nucleic acid
Collar
Sheath
Baseplate
Spikes
Tail fiber

LIFECYCLE:

1.**Adsorption:**

- The first step in the infection process is the adsorption of the phage to the bacterial cell.
- This step is mediated by the tail fibers or by some analogous structure on those phages that lack tail fibers.
- Phages attach to specific receptors on the bacterial cell such as proteins on the outer surface of the
- bacterium, LPS, pili, and lipoprotein
- This process is reversible. One or more of the components of the base plate mediates irreversible binding of phage to a bacterium.

2.Penetration:

- The irreversible binding of the phage to the bacterium results in the contraction of the sheath
- (for those phages which have a sheath) and the hollow tail fiber is pushed through the bacterial envelope.
- Some phages have enzymes that digest various components of the bacterial envelope
- Nucleic acid from the head passes through the hollow tail and enters the bacterial cell.
- The remainder of the phage remains on the outside of the bacterium as “ghost”.
- Even a non-susceptible bacterium can be artificially infected by injecting phage DNA by a process known as transfection..

- Depending on the life cycle, phages can either by lytic (virulent) or lysogenic (temperate).
- While lytic phages kill the cells they infect, temperate phages establish a persistent infection of the cell without killing it.
- In lytic cycle the subsequent steps are synthesis of phage components asembly, maturation and release.

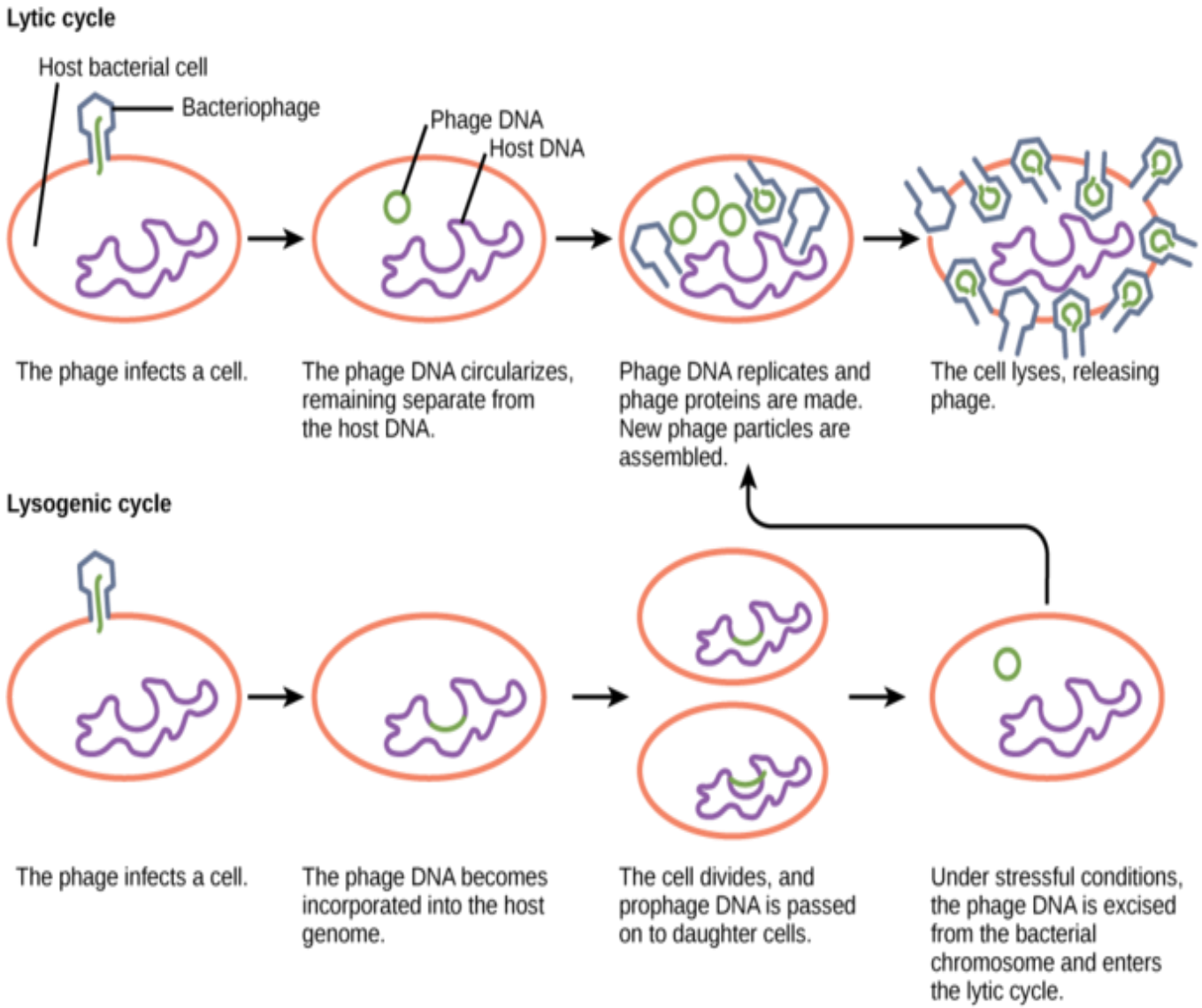
Lytic cycle:

- Lytic or virulent phages are phages, which multiply in bacteria and kill the cell by lysis at the end of the life cycle.
- Soon after the nucleic acid is injected, the phage cycle is said to be in eclipse period.
- During the eclipse phase, no infectious phage particles can be found either inside or outside the bacterial cell.
- Eclipse phase represents the interval between the entry of phage nucleic acid into bacterial cell and release of mature phage from the infected cell.
- This phase is devoted to synthesis of phage components and their assembly into mature phage particles.

- The phage nucleic acid takes over the host biosynthetic machinery and phage specified m-RNA's and proteins are made.
- In some cases the early phage proteins actually degrade the host chromosome.
- Structural proteins (head, tail) that comprise the phage as well as the proteins needed for lysis of the bacterial cell are separately synthesized.
- Nucleic acid is then packaged inside the head and then tail is added to the head. The assembly of phage components into mature infective phage particle is known as maturation.
- In Lysis and Release Phase the bacteria begin to lyse due to the accumulation of the phage lysis protein and intracellular phage are released into the medium.
- It is believed that phage enzymes weaken the cell wall of bacteria.
- The number of particles released per infected bacteria may be as high as 1000.
- The average yield of phages per infected bacterial cell is known as burst size.

Lysogenic cycle:

- Lysogenic or temperate phages are those that can either multiply via the lytic cycle or enter a dormant state in the cell.
- In most cases the phage DNA actually integrates into the host chromosome and is replicated along with the host chromosome and passed on to the daughter cells.
- This integrated state of phage DNA is termed prophage.
- This process is known as lysogeny and the bacteria harboring prophage are called lysogenic bacteria.
- Since the prophage contains genes, it can confer new properties to the bacteria.
- When a cell becomes lysogenized, occasionally extra genes carried by the phage get expressed in the cell. These genes can change the properties of the bacterial cell.
- This process is known as lysogenic conversion or phage conversion.


Lytic cycle
Host bacterial cell
Bacteriophage
Phage DNA
Host DNA
The phage infects a cell.
The phage DNA circularizes, remaining separate from the host DNA.
Phage DNA replicates and phage proteins are made. New phage particles are assembled.
The cell lyses, releasing phage.
Lysogenic cycle
The phage infects a cell.
The phage DNA becomes incorporated into the host genome.
The cell divides, and prophage DNA is passed on to daughter cells.
Under stressful conditions, the phage DNA is excised from the bacterial chromosome and enters the lytic cycle.

ISOLATION OF PURE CULTURE TECHNIQUES

- **Culture** : Act of cultivating microorganisms or the microorganisms that are cultivated.
- **Mixed culture** : more than one microorganism
- **Pure culture** : containing a single species of organism.
- A pure culture is usually derived from a mixed culture (one containing many species) by transferring a small sample into new, sterile growth medium in such a manner as to disperse the individual cells across the medium surface or by thinning the sample many times before inoculating the new medium.

- Pure cultures are important in microbiology for the following3 reasons..
- 1.Once purified, the isolated species can then be cultivated with the knowledge that only the desired microorganism is being grown.
- 2. A pure culture can be correctly identifiedfor accurate studyingand
- testing, and diagnosis in a clinical environment.
- 3.Testing/experimenting with a pure culture ensures that the same results can be achieved regardless of how many time the test is repeated.

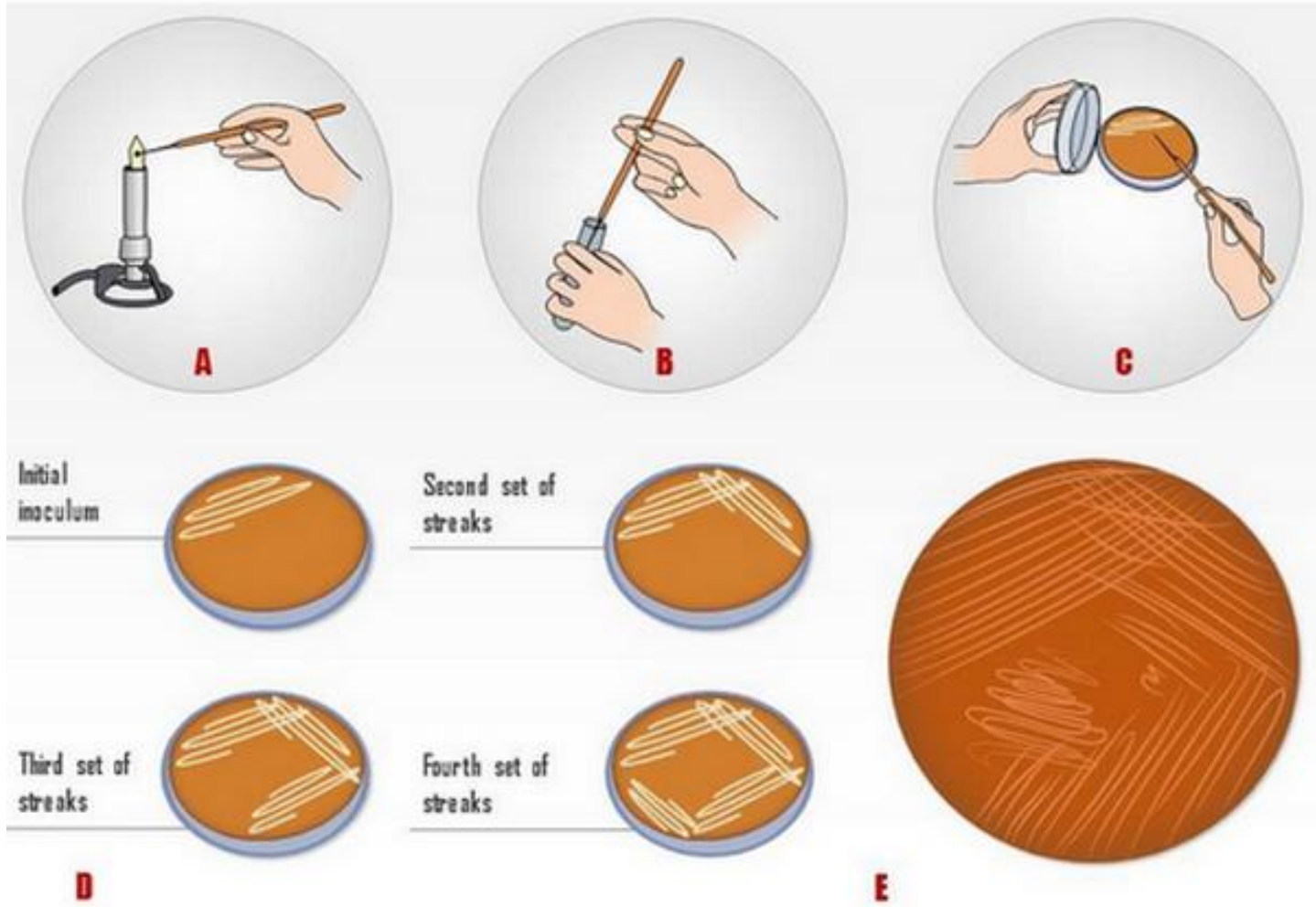
Common isolation techniques

- Streak plate method
- Pour plate method
- Spread plate method
- Serial dilution method
- Single cell isolation method
- Enrichment culture method
- Sweep plate method
- Stab culture
- Lawn/ Carpet method
- Roll tube method

1.Streak plate method:

- Streaking is the process of spreading the microbial culture with an inoculating needle on the surface of themedia.
- Sterilizethe inoculating needle by flame to make redhot and allow it to coo lfor 30 seconds.
- The sample is streaked in such a way to provide seeries of dilution.
- purpose-thin out innoculum to get seprate colonies.
- subculturing can be done by streaking isolated colonies from streakplatet on new plate.

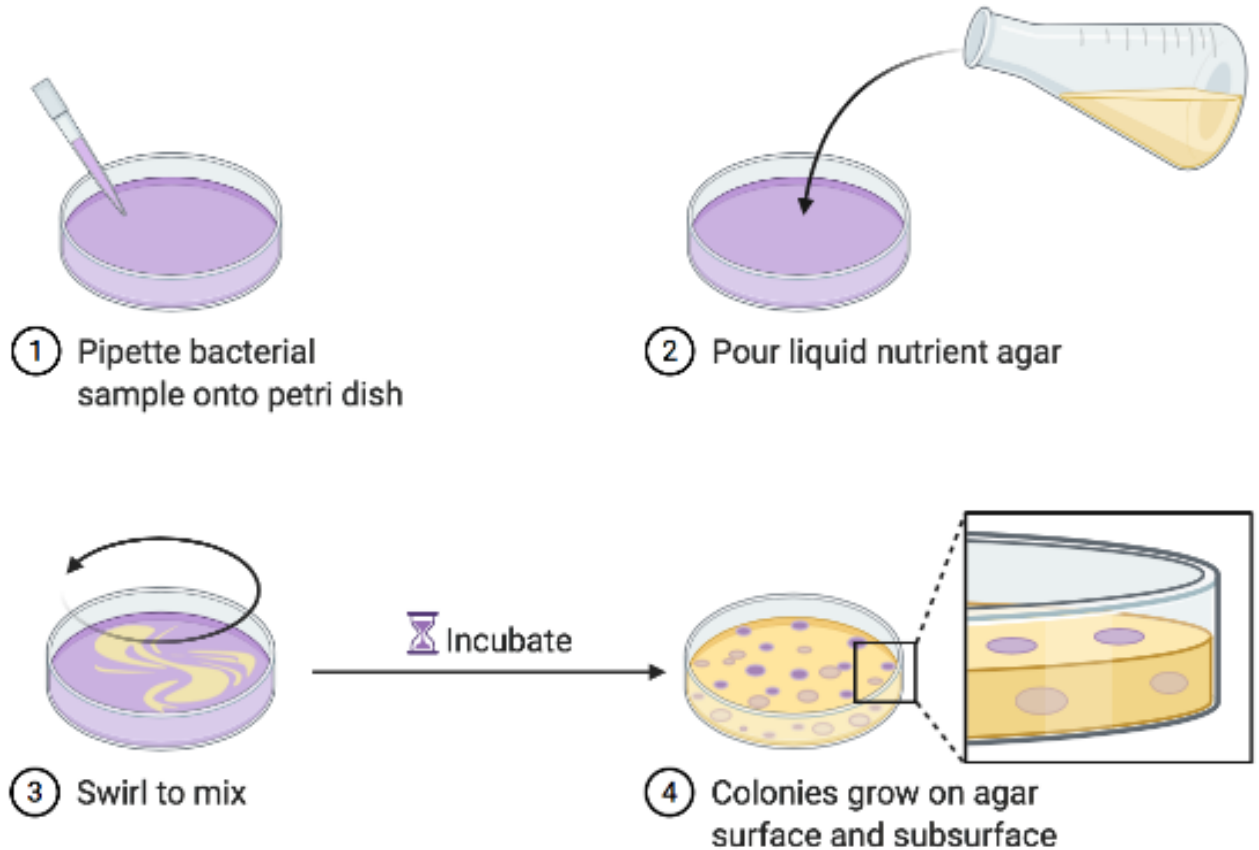
The Streak Plate Isolation Method



2. Pour plate method:

- Thebacterial culture andliquid agarmedium aremixedtogether.
- After mixing the medium,the medium containing the culture poured into sterilizedvpetridishes (petriplates), allowed solidifying and then incubated.
- After incubation colonies appear on the surface
- **DISADVANTAGES**
- 1.Microorganism trapped beneath the surface of medium hence surface as well as subsurface colonies are developed which makes the difficulties in counting the bacterial colony.
- 2. Tidious and time consumingg method, microbes are subjected to heat shock because liqiudmediummaintainedat45℃.
- 3. Unsuitable-Psychrophile

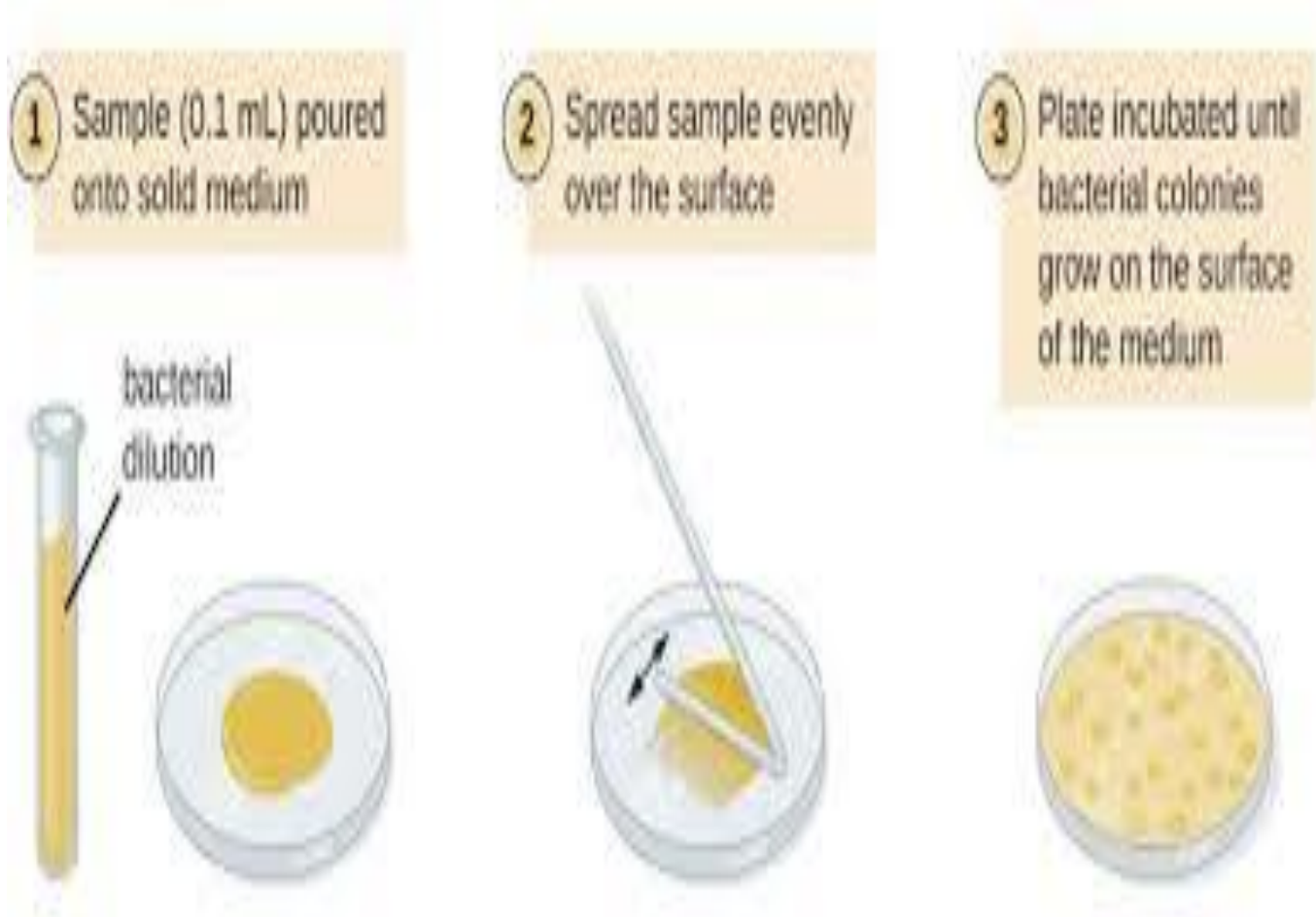
Pour Plate Method



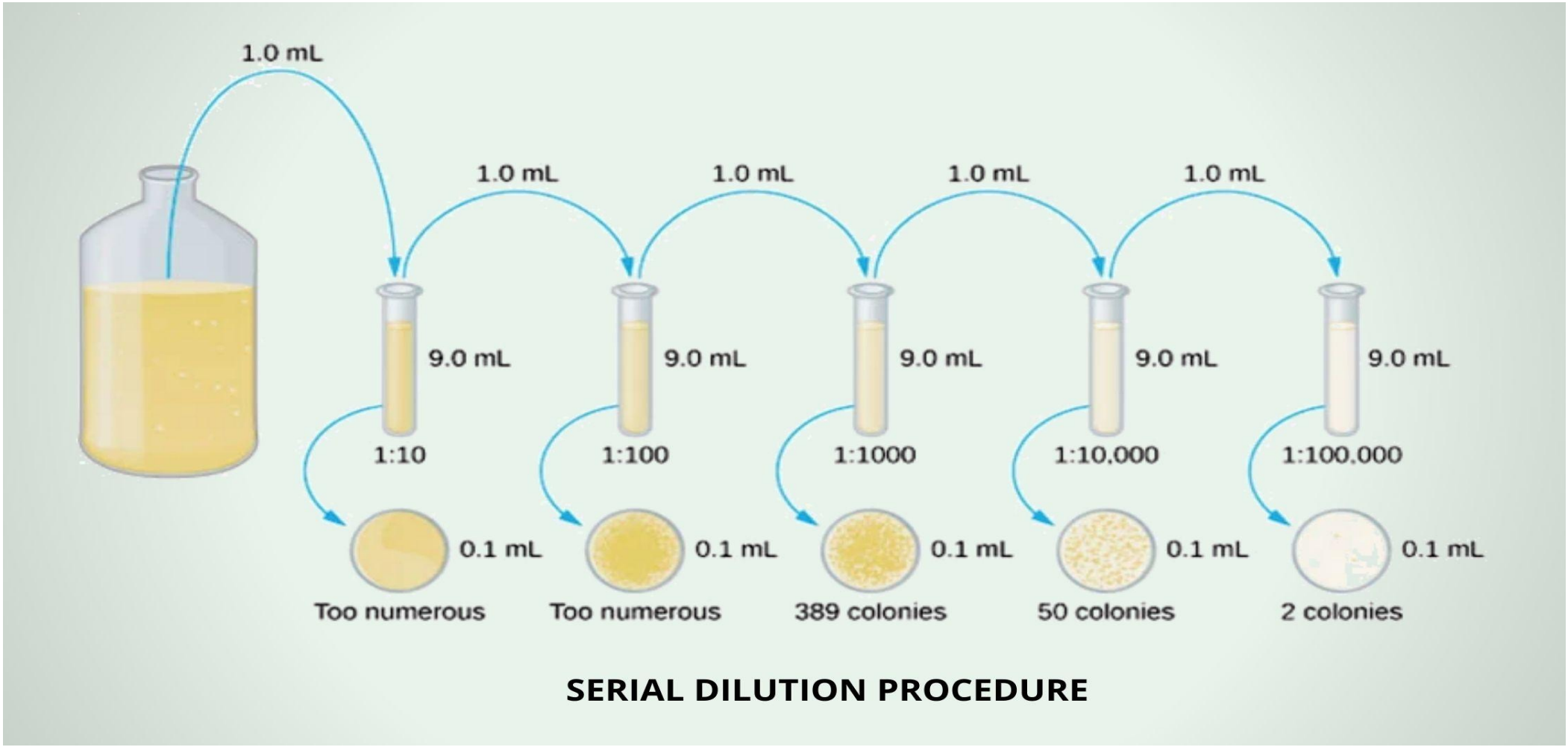
3. Spread plate method:

This is the best method to isolate the pure colonies.

- In this technique, the culture is not mixed with the normal saline andserialy diluted.
- 0.1 ml of sample taken from diluted mixture, which is placed on the surface of the agar plate and spread evenly over the surface by using L shaped glassrod called spreader.
- Incubate theplates. After incubation, colonies are observed on the agar surface.

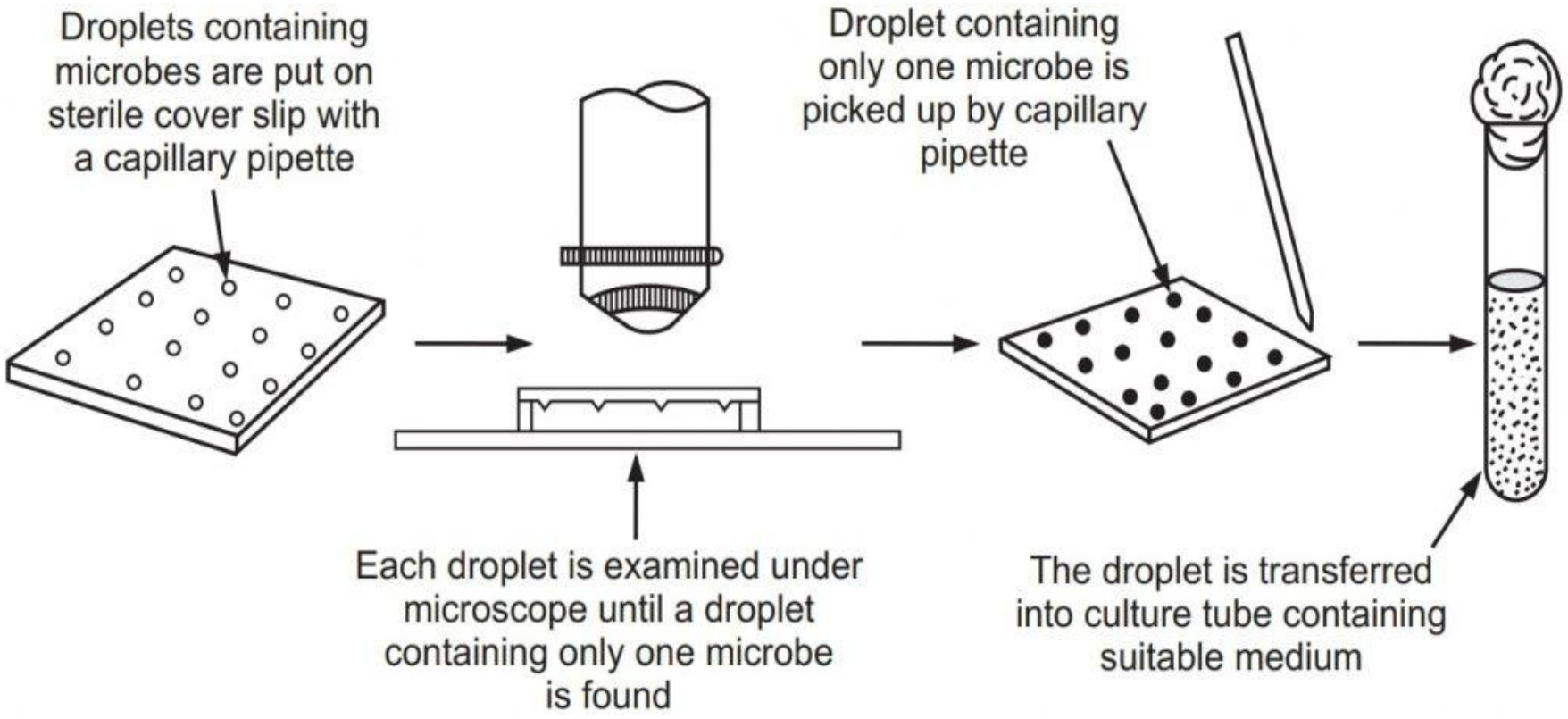

1 Sample (0.1 mL) poured onto solid medium
2 Spread sample evenly over the surface
3 Plate incubated until bacterial colonies grow on the surface of the medium
bacterial dilution

Dilution plating/ serial dilution



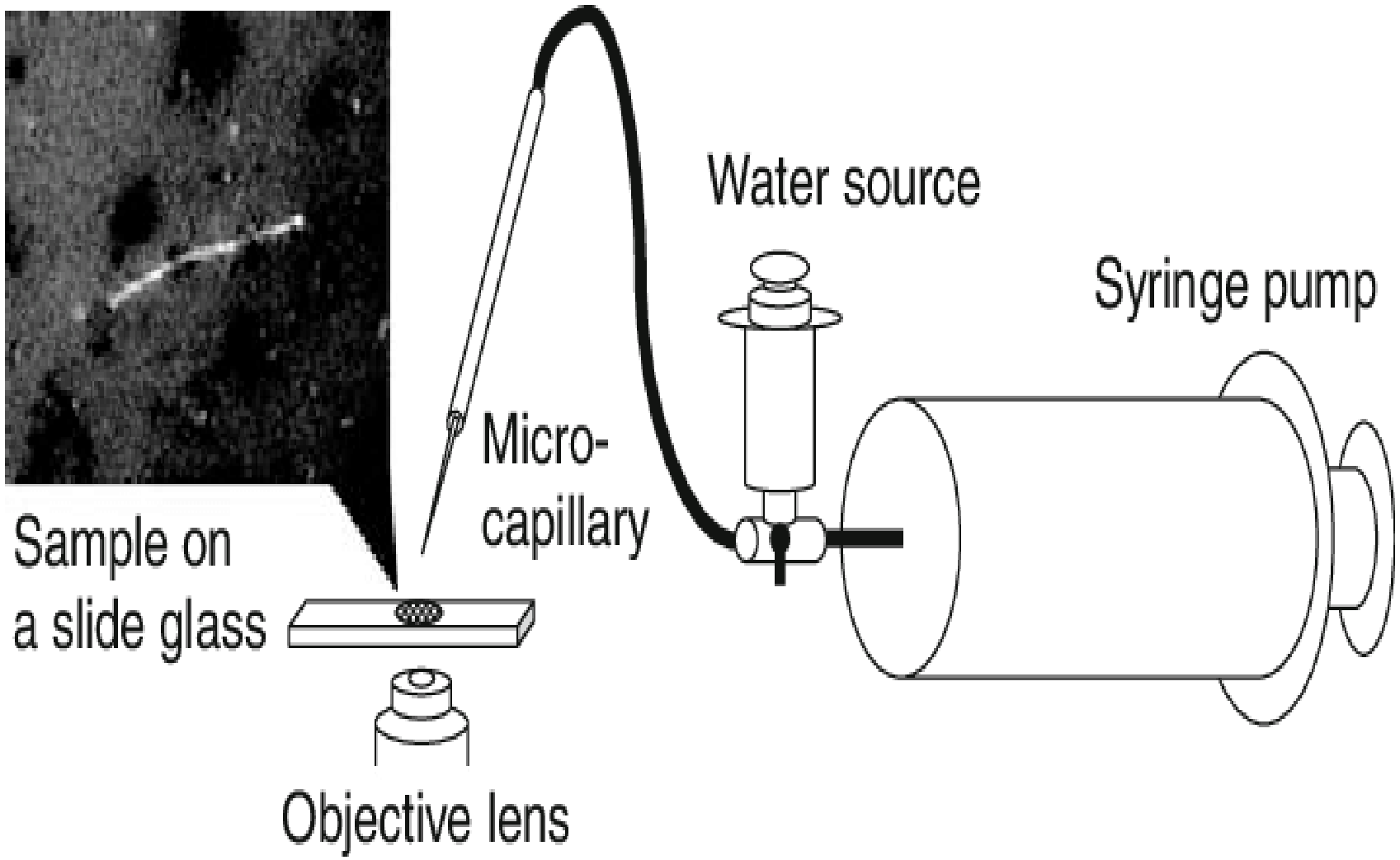
Single cell isolation methods:

- 1.Capillary pipette method:

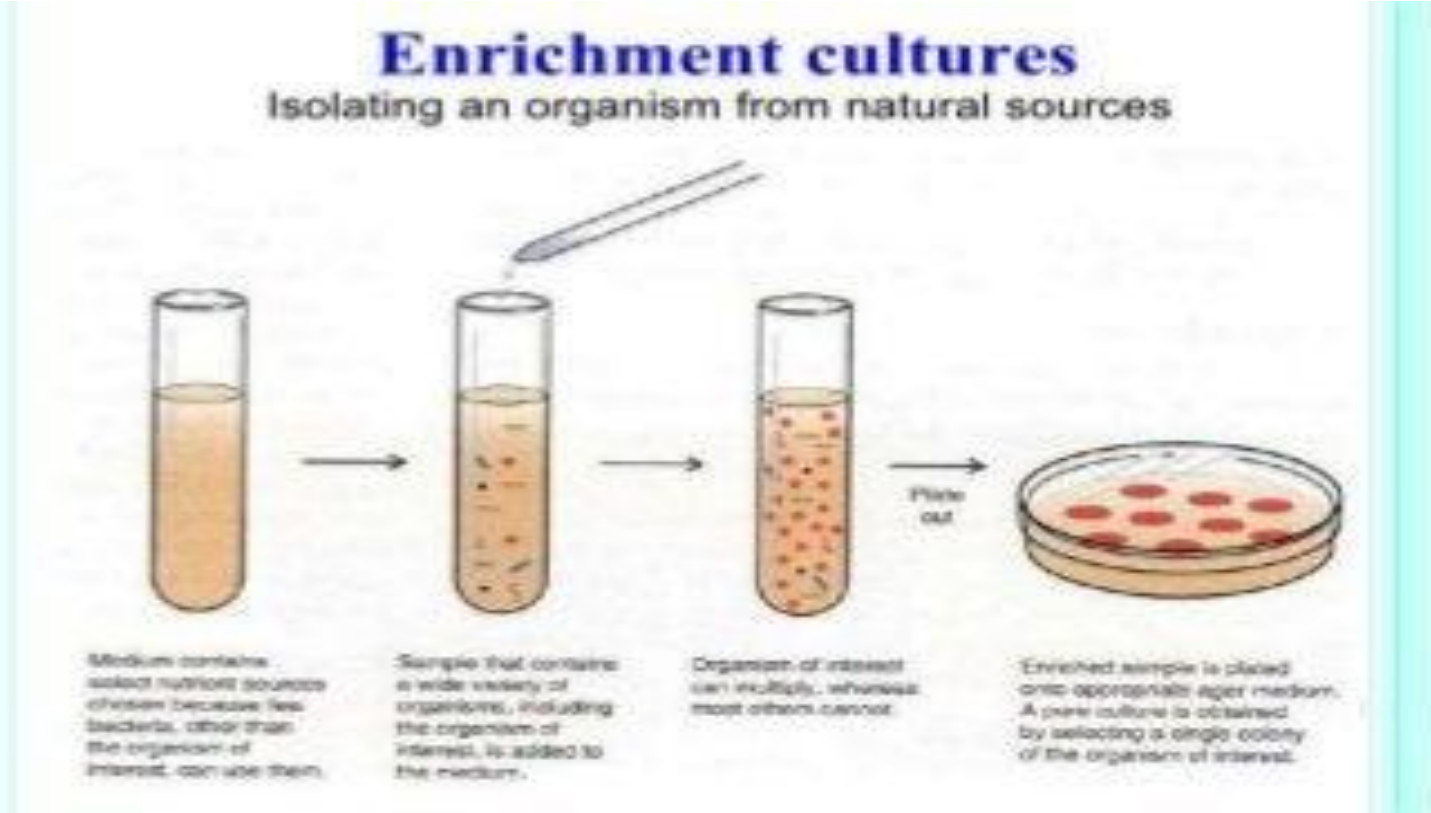


2.Micromanipulator method:

- Micromanipulators have been built, which permit one to pick out a single cell from a mixed culture.
- This instrument is used in conjunction with a microscope to pick a single cell (particularly bacterial cell) from a hanging drop preparation.
- the single cell of microbe sucked into micropipette and transfered to large amount of sterile medium.
- ADVANTAGES OF MICROMANIPULATOR METHOD: The advantages of this method are that one can be reasonably sure that the cultures come from a single cell and one can obtain strains with in thespecies.
- DISADVANTAGESThe disadvantages are that the equipment is expensive,its
- manipulation is very tedious, and it requires a skilled person.

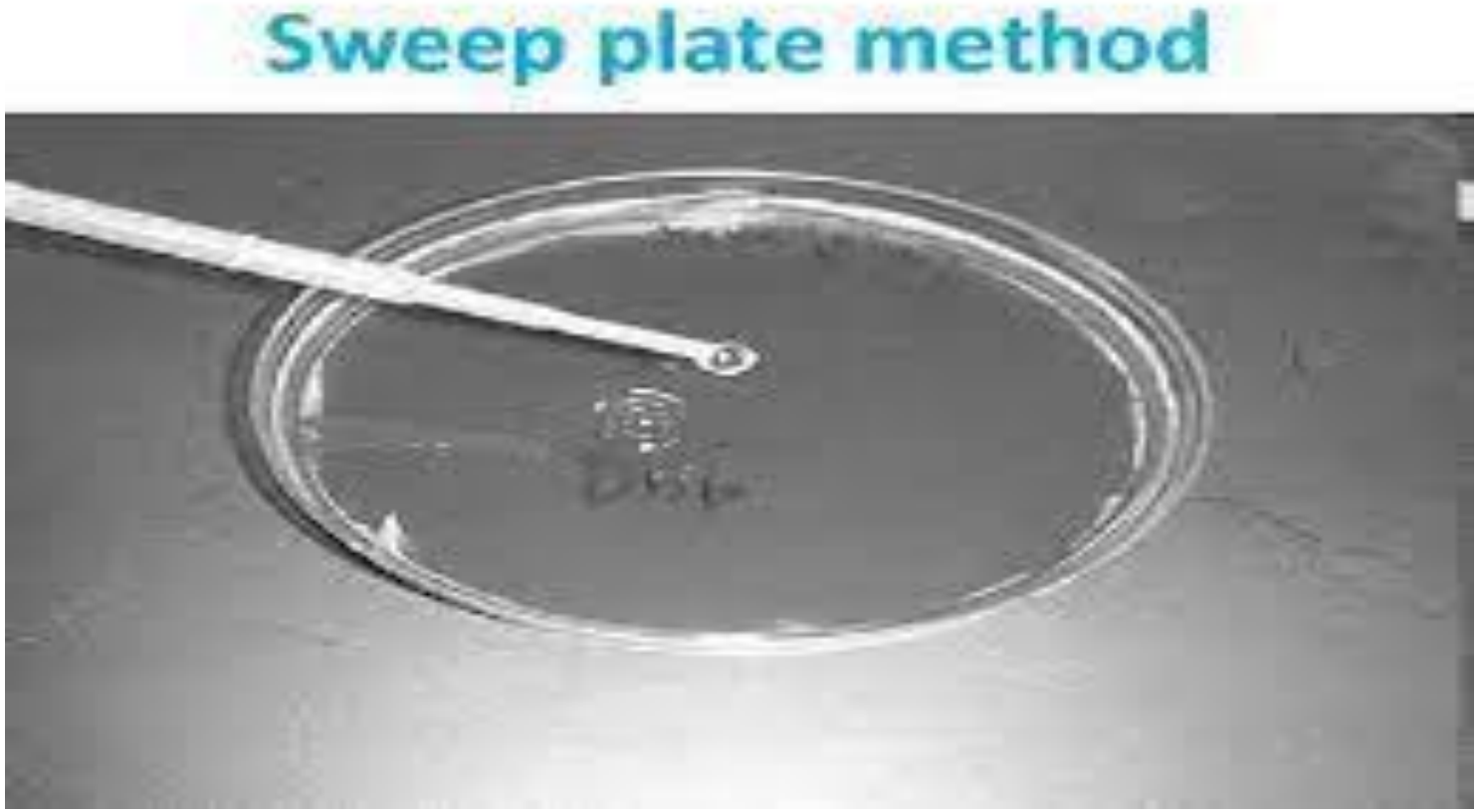

Water source
Syringe pump
Micro-
capillary
Sample on
a slide glass
Objective lens

Enrichment culture method



Roll tube method

Sweep plate method



Stab culture:

Lawn/ carpet culture

Liquid media

PRESERVATION OF PURE CULTURE:

- T o maintain pure culture for extended periods in viable condition without any genetic change is referred as Preservation.
- During preservation most important factor is to stop microbial growth or at least lower the growth rate.
- D u e to this toxic chemicals are notaccumulated and hence viability of accumulated and hence viability of micro org is not effected.

- Objectives of preservation:

- 1. To maintain isolated pure culture for extended periods in a viable conditions.
- 2. To avoid contamination
- 3. To restrict Genetic Mutation

Preservation methods of Bacteria:

- 1. Periodic transfer to fresh medium.
- 2. Storage at low temprature
- 3. Storage in sterile soil
- 4. Preservation by overlaying culture with mineral oil
- 5. Lyophillization or freeze drying

1. Periodic transfer to fresh medium

- Strains can be maintained by periodically preparing a fresh interval at which the culture from the previous stock culture.
- The culture medium, the storage temperature, and the time transfers are made vary with the species.
- The temperature and the type of medium chosen should support a slow rather than a rapid rate of growth so that the time interval between transfers can be as long as possible.
- Many of the more common heterotrophs remain viable forr several weeks or monthson a medium like NutrientAgar.
- The transfer method has the disadvantage of failing to prevent changes in the characteristics of a strain due to the development of variants and mutants

2. Storage at low temprature

- 1. REFRIGERATION
- 2. CRYOPRESERVATION

REFRIGERATION

- Pure cultures can be successfully stored at 0-4°C either in refrigerators or in cold-rooms.
- ❖ This method is applied for short duration (2-3 weeks for bacteria and 3-4 months for fungi) because the metabolic activities of the microorganisms are greatly slowed down but notstopped.
- ❖ Thus their growth continue slowly, nutrients are utilized and waste products released in medium.
- ❖ This results in finally the death of the micro

2. CRYOPRESERVATION

- ❖ Cryopreservation (i.e., freezing in liquid nitrogen at -196°C or in the gas phase above the liquid nitrogen at -150°C) helps survival of pure cultures for long storage times.
- ❖ In this method, the microorganisms of culture are rapidly frozen in liquid nitrogen at -196°C in the presence of stabilizing agents such as glycerol or Dimethyl Sulfoxide (DMSO) that prevent the cell damage due to formation of ice crystals and promote cell survival.
- ❖ This liquid nitrogen method has been successful with many species that cannot be

preserved by lyophilization and most species can remain viable under these conditions for

10 to 30 years without undergoing change in their characteristics, however this method is expensive.

4.Preservation by overlaying culture with mineral oil:

- 1. This is a simple and most economical method of maintaining pure cultures.
- 2. In this method, sterile liquid paraffin is poured over the slant (slope) of culture and stored upright at room temperature. The layer of paraffin ensures anaerobic conditions and prevents ddehydration of the medium.
- 3. This condition helps microorganisms or pure culture to remain in a dormant state and, therefore, the culture can be preserved form months to years (varies with species).
- ADVANTAGES
- 1. We can remove some of the growth under the oil with a transfer needle, inoculate a

fresh medium, and still preserve the original culture. The simplicity of the method makes it attractive, but changes in the characteristics of a strain can stilloccur.

5. Lyophillization or freeze drying

- Freeze drying is a stabilizing process in which a substance is first frozen and then the quantity of the solvent is reduced, first by sublimation (primary drying stage) and then desorption (secondary drying stage) Better preservation occurs with freeze-drying thanwith other methods because freeze-drying reduces the risk of intracellular ice crystallization that compromises viability
- Removal of water from the specimen effectively prevents this damage
- Lyophilization is greatest with gram-positivebacteria (spore formers) and decrese with gram -negative bactetia but viability can be maintained as long as 30 yearsLarge numbers of vials of dried microorganisms can be stored with limited space, and organisms can be easily transported long distances at room temperature 30
- The process combines freezing and dehydration- Organisms are initially frozen and then dried by lowering the atmospheric presssure with a vacuumapparatus Specimens can be connected individually to the condenser (manifold method) or can be placed (in a chamber) where they are dehydrated in one larger airspace