Microbial world

(Prions, Mycoplasma, Phytoplasma, Actinomycetes, Plasmids)

BBZ

Botany

Semester I

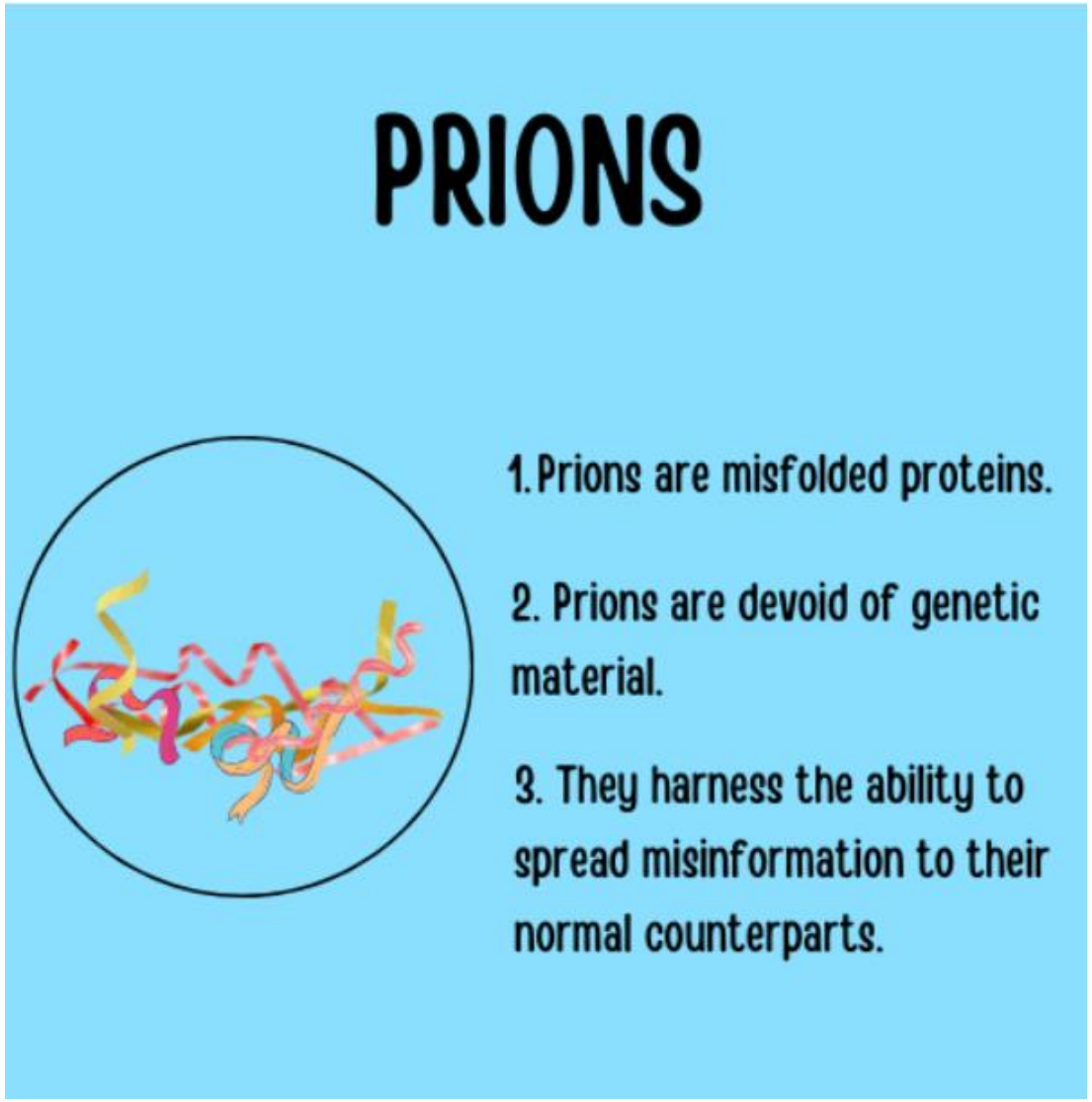
PRIONS

1957: Caleton Gajciusek discovered the infectious agent in new Guinea

Responsible for many degenerative diseases.

Once a misfolded prion enters a healthy person potentially by eating infected food, it converts correctly folded protein into disease associated form.

Mechanism of action unclear



Mycoplasma

INTRODUCTION

- ***Mycoplasmas* are the smallest and simplest self-replicating bacteria.**
- The mycoplasma cell contains minimum set of organelles essential for growth and replication: a plasma membrane, ribosomes, and a genome consisting of a double-stranded circular DNA molecule.
- Unlike all other prokaryotes, **the mycoplasmas have no cell walls, and they are consequently placed in a separate class *Mollicutes*(*mollis*, soft; *cutis*, skin).**

CLASSIFICATION

Kingdom	:	Bacteria
Division	:	Firmicutes
Class	:	Mollicutes
Order	:	Mycoplasmatales
Family	:	Mycoplasmataceae
Genus	:	*Mycoplasma*

Classification of Mycoplasma

Mycoplasmas have been placed in the

Division. Tenericutes

Class-Mollicutes (literally meaning soft skin),

Order –Mycoplasmatales

The order-Mycoplasmatales contains the following families and genus

1. Family-**Mycoplasmataceae**, to which parasitic mycoplasmas belong and requiring cholesterol or other sterols as an essential growth factor. This contain two genera

    (a) Genus-**Mycoplasma** which utilises glucose or arginine but does not split urea, and

    (b) Genus-**Ureaplasma** which hydrolyses urea.

2. Family-**Acholeplasmataceae**, mostly saprophytic mycoplasmas, which do not require sterols as growth factor;

    genus-**Acholeplasma**.

3. Family-**Spiroplasmataceae**; containing the genus **Spiroplasma** which parasitise arthropods and plants. They are sterol dependent. These are helical in shape.

Some genera are of uncertain taxonomic position such as genu **Anaeroplasma** and genus-**Asteroleplasma**.

General Characters of Mycoplasmas

The mycoplasmas have the following main characteristics

1. **They are very small, unicellular, usually non-motile, microorganisms.**
2. **They can be grown in cell free media forming typical 'fried-egg' shaped colonies**
3. **They are highly pleomorphic, varying with culture conditions.**

coccoid bodies
asteroid bodies
Ring forms
Fine filaments which may be branched

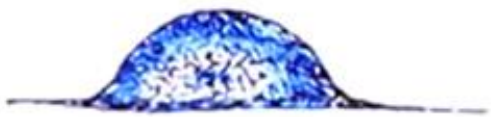
‘JOKERS OF PLANT KINGDOM’

4. **They are filterable through bacterial filters.**
5. **Cell wall is absent. Cells are bounded by a triple layer unit membrane**
6. **Both DNA and RNA are found in mycoplasma.**
7. **They are highly resistant to penicillin but are inhibited by tetracycline**
8. **They are mostly free living, parasites and saprophytes.**
9. **Chemically they have 4 per cent DNA and 8 per cent RNA.**
10. **They lack cell wall precursors like muramic acid or diaminopimelic acid**

Mycoplasma : Morphology

Entire colony

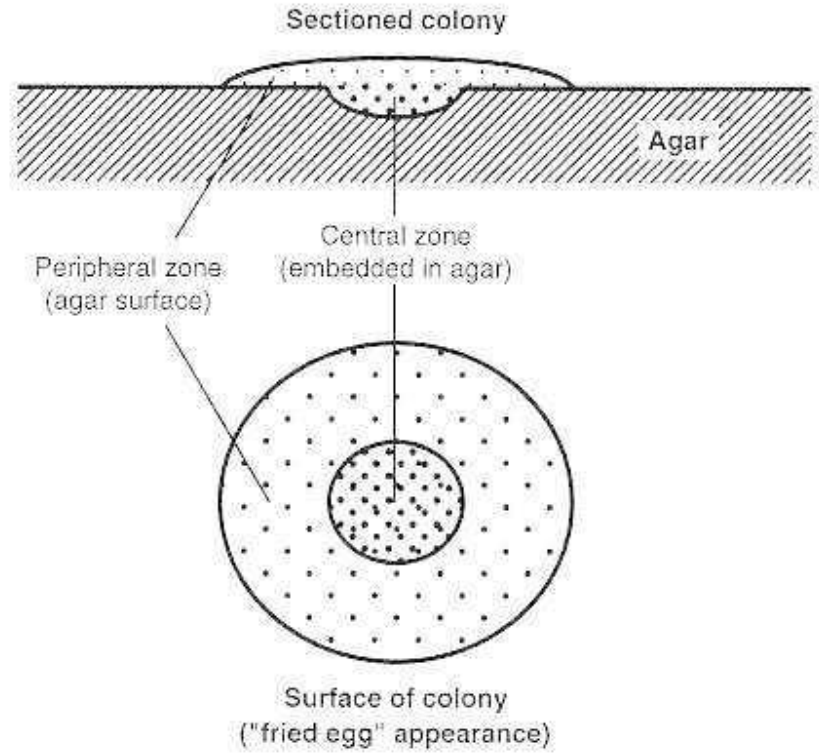
Spherical form



L.S. of a colony growing on agar surface

Polymorphic forms

Irregular filamentous form



History

- Discovered by Pasture in 1843.
- Isolated from pleural fluid of cattle.
- It was called as *Mycoplasma mycoides*.
- Nocard & Roux first cultured *Mycoplasma* in1898.
- In 1929, Nowak called them PPLO.

Introduction

- Unicellular Prokaryote
- Gram-Positive Bacteria
- Lack cell wall- Pleomorphic
- Osmotic Shock / Detergent-Sensitive
- Antibiotics-Tetracyclin / Chloremphenicol-Sensitive
- They are the smallest bacteria capable of autonomous growth

- Facultative anaerobes / obligate anaerobes
- Low G+C%, 23-41%
- Mostly require sterol for their growth
- (but some genera do not require sterol – such as *Acholeplasma, Asteroleplasma, Mesoplasma)*

Structure of Mycoplasmas:

The cell is devoid of cell wall which makes them readily deformable showing irregular and variable shapes. They may be ring-like, granular, coccoid, pear-shaped, filamentous, etc. (Fig. 2.50). The filaments are of two types: unbranched or branched.

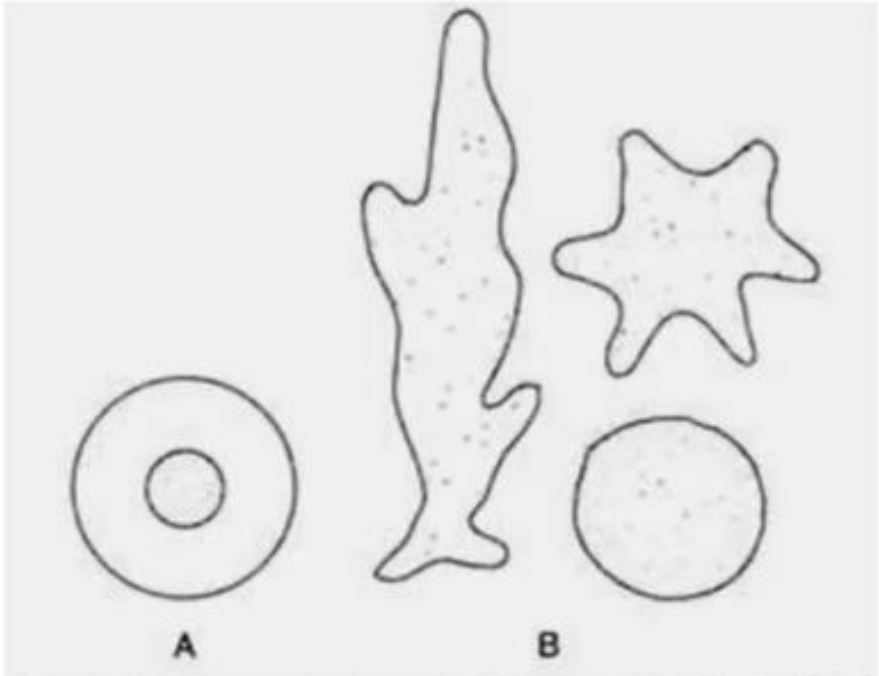
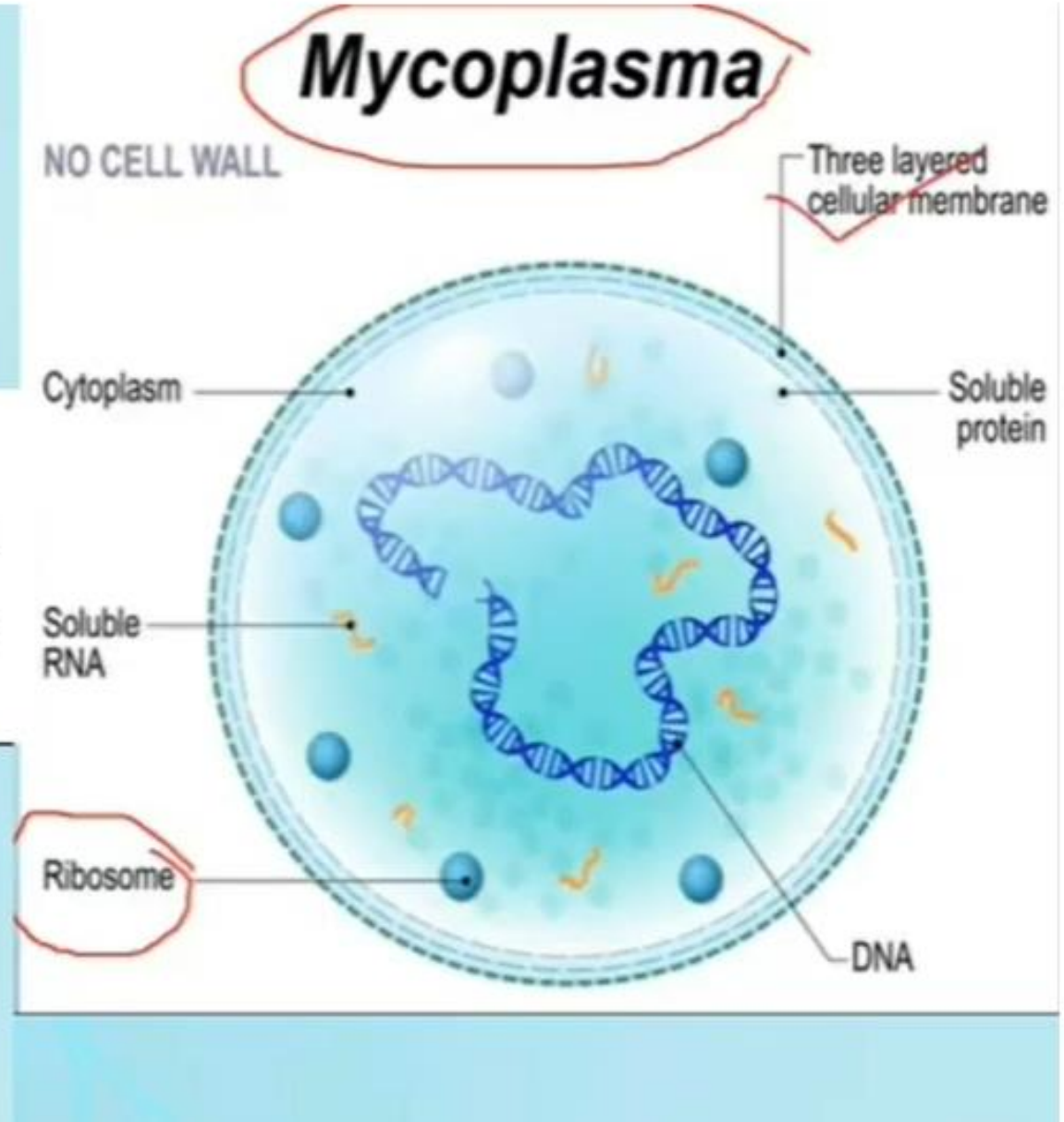
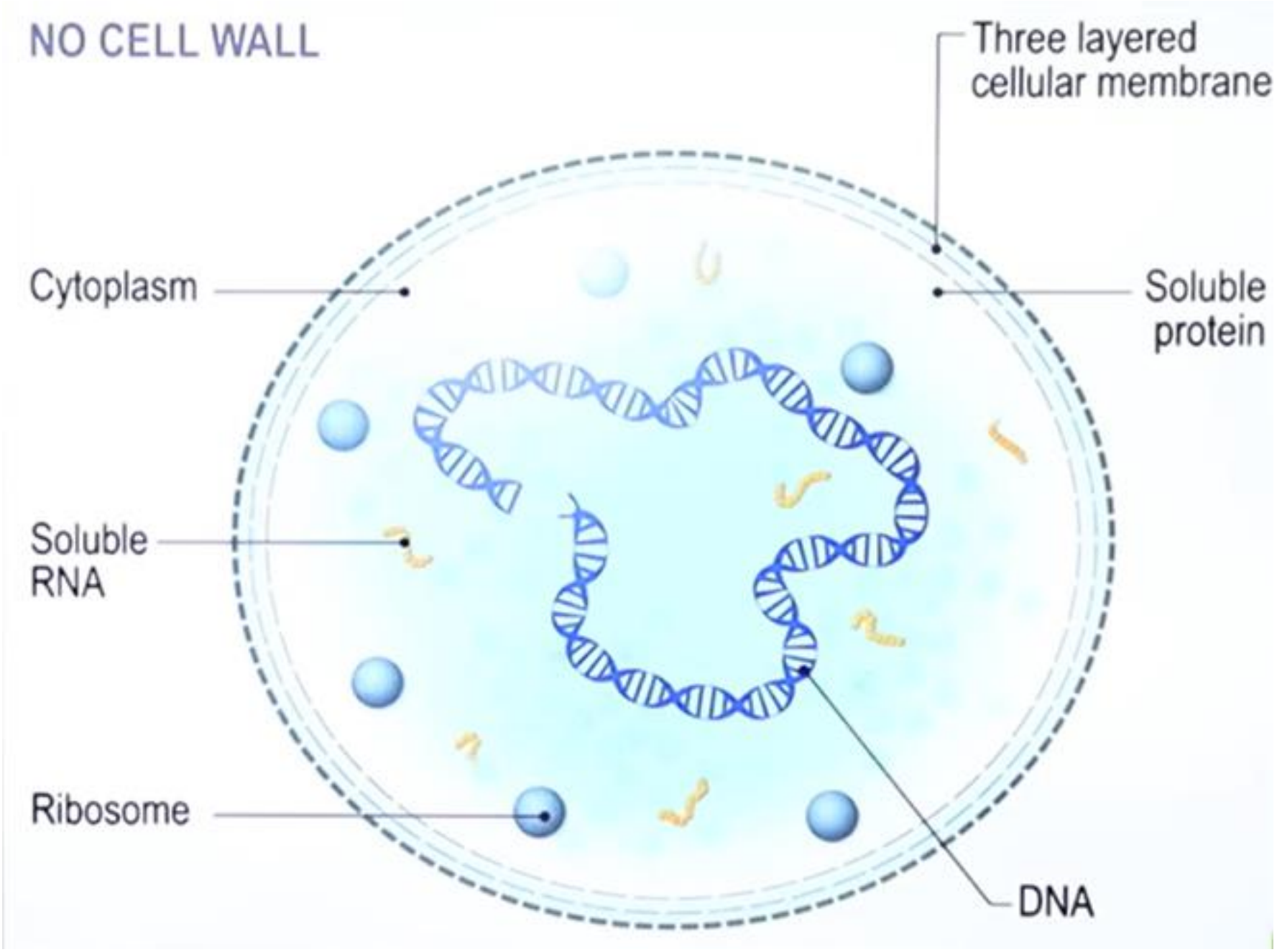


Fig. 2.50 : Mycoplasmas : A. Single colony, B. Cells of various shapes

The cells are covered by cytoplasmic (lipoprotein) membrane (Fig. 2.51). Cell membrane covers the cytoplasm which contains nucleoplasm like structure and ribosomes. The genetic material is composed of DNA and RNA. It is about less than 50%, the amount present in other prokaryotic organisms. The amount of RNA (8%) is more than DNA (4%).



Structure of Cell



Chemical Composition

- Protein - 40-60%
- Carbohydrate - 0.1%
- DNA Content - 3-7%
- Lipid - 8-20%

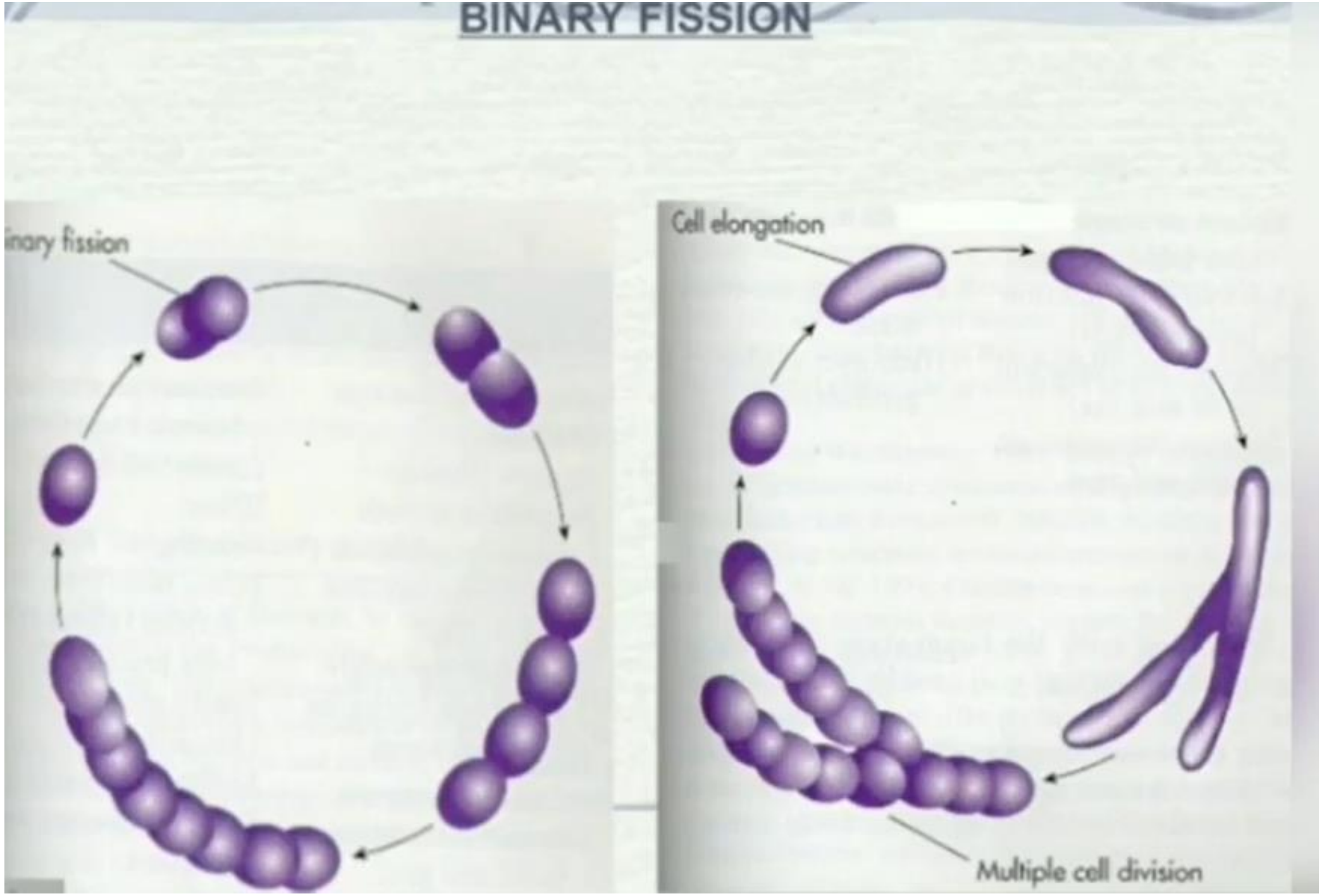
REPRODUCTION IN MYCOPLASMA

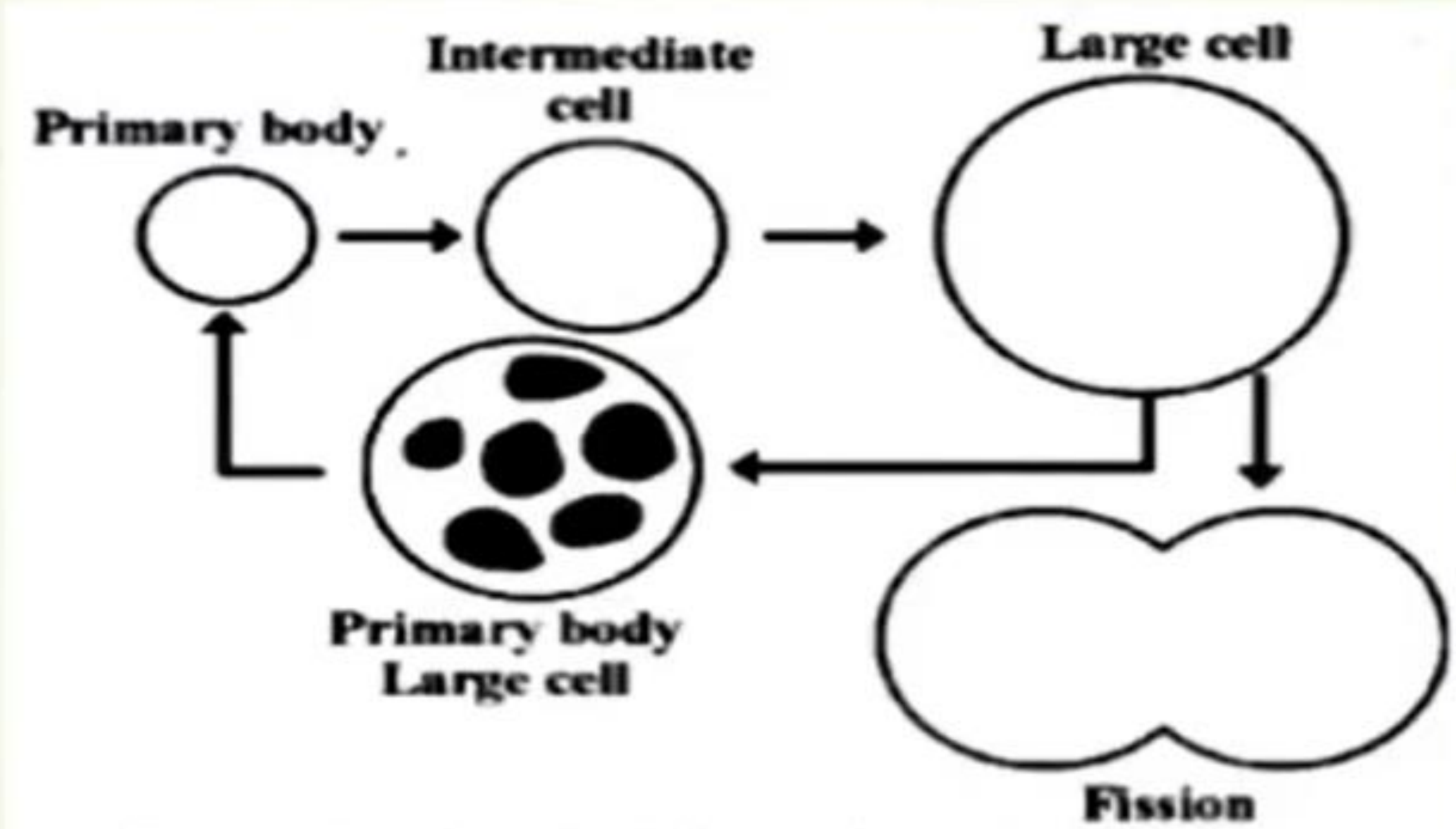
- According to Morowitz and Tourtelotte(1962) sexual reproduction is absent in mycoplasma.

Common methods of reproduction are-

- Binary Fission
- Elementary Bodies

BINARY FISSION





Reproduction in Mycoplasma

Pathogenesity

- Extra cellular parasite
- It Causes infection in Respiratory tract & Urinogenital tract
- In Respiratory tract Cause Pneumonia
- In Urinogenital tract Cause Male sterility

Penetration:-

- Mucosa membrane penetrate
- Attach on epithelial cells
- Growth on epithelial cells
- And destroy epithelial cells
- Cause Pneumonia

Treatment:-

- Tetracyclin / Chloramphenicol / Erythromycin

Transmission of Disease:

- By Leaf hopper, plant hopper
- By grafting
- By Parasitic Haustoria
- By Aphids eg. Acrythosiphon pisum.

Mycoplasmal Plant Diseases

- Little Leaf of Brinjal
- Stripe Disease of Sugarcane
- Bunchy Top of Papaya
- Yellow Dwarf of Tomato
- Corn Stunt Disease
- Yellow Disease of Vinca
- Virescence in Cotton and Clover
- Big Bud of Tomato
- Witches top of Papaya
- Clover Phyllody Disease

Mycoplasmal Human Disease :

- Respiratory Disease ....**M. pneumoniae, M. salivarium**
- Inflammation of Genitals.....**M. huminis**
- Infertility ..... **M. fermentans**

Phytoplasma

1. Phytoplasma are unicellular prokaryotic organism or phytopathogens.
2. They are small in size ranging between 200-800 nm. Phytoplasmas are obligate bacterial parasites of plant phloem tissue and of the insect vectors that are involved in their plant to plant transmission. Phytoplasma were discovered in 1967 by Japanese Scientists, who termed it as MLO.
3. Lack cell wall, only triple layered lipoprotein plasma membrane present.
4. Phytoplasma show the polymorphism. Phytoplasma cannot grown on artificial media making it difficult to study their biology
5. Phytoplasmas are generally ovoid but filamentations forms have been exceptionally observed.
6. Genetic material of phytoplasma contains both DNA and RNA. They are sensitive to tetracyclines and insensitive to penicillins.
7. Phytoplasma transmitted by vectors, grafting, dodder or insects.
8. Phytoplasmas are known to have smallest genome of all the living organisms.
9. Disease caused by phytoplasma as little leaf of egg plant.

Conclusion

- *Mycoplasma* are peculier type of bacteria
- as they requiring sterol for growth
- They are cell wall less
- some are pathogenic (humen diseses)
- some are cattle/plants/insects parasite also

Actinomycetes/Actinobacteria

Actinomycetes are Gram positive, rod shaped and spore forming bacteria.

Actinomycetes referred as connective link between bacteria and fungi because they form aerial filaments like fungi (filaments are smaller than fungi filaments).

They can be both aerobic (filamentous form) and anaerobic in nature.

Actinomycetes are most abundant in soil and gives distinctive earthy smell.

Actinomycetes/Actinobacteria

Actinomycetes fix nitrogen in soil via having symbiotic relationship with plants.

Actinomycetes decompose organic material and enrich soil which is used as compost.

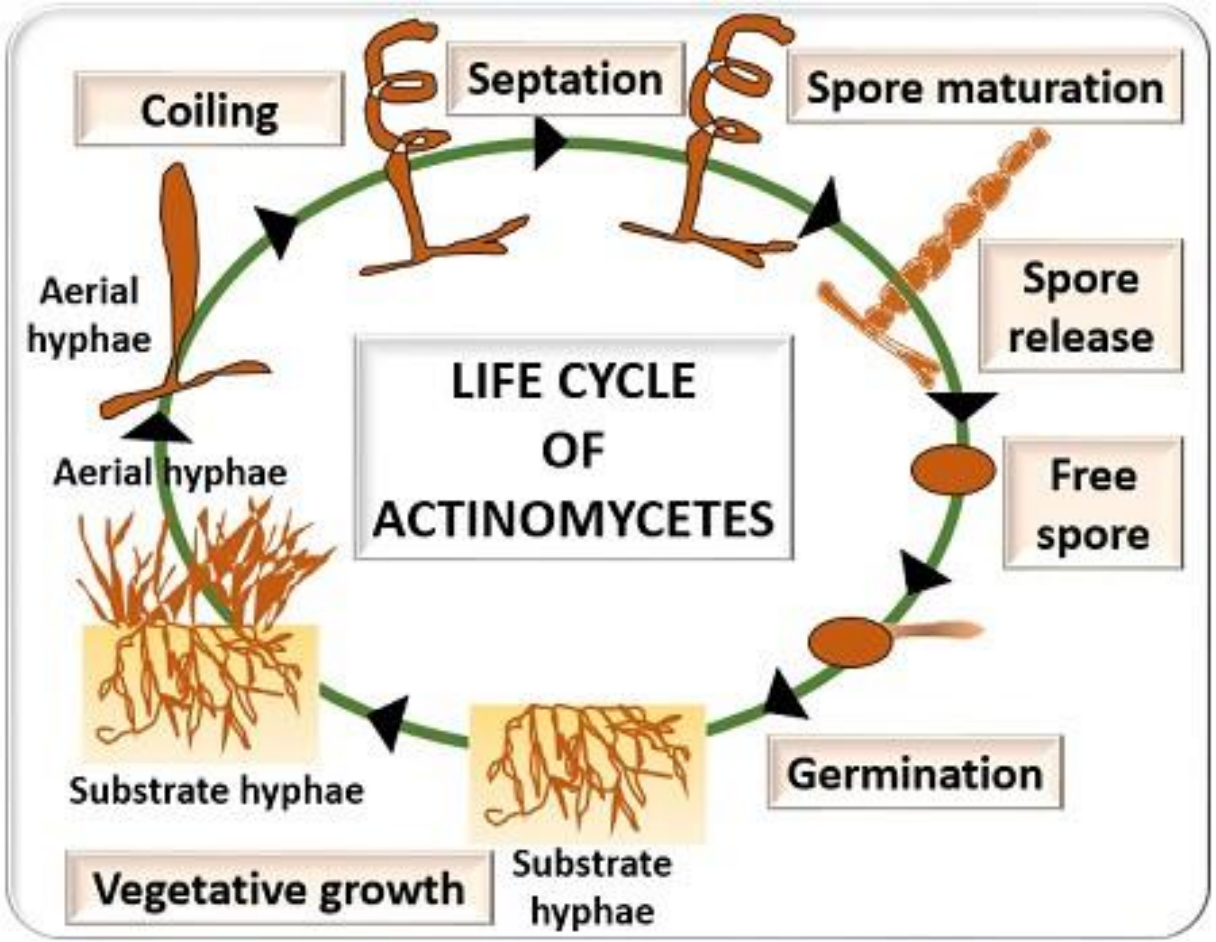
Many antibiotics like streptomycin, erythromycin, neomycin are obtained from Actinomycetes.

Genus *Actinomycetes* are common member of human oral cavities and occasionally cause infection in oral mucosa. *Nocardia asteroids* which is commonly present in soil can cause respiratory tract infections.

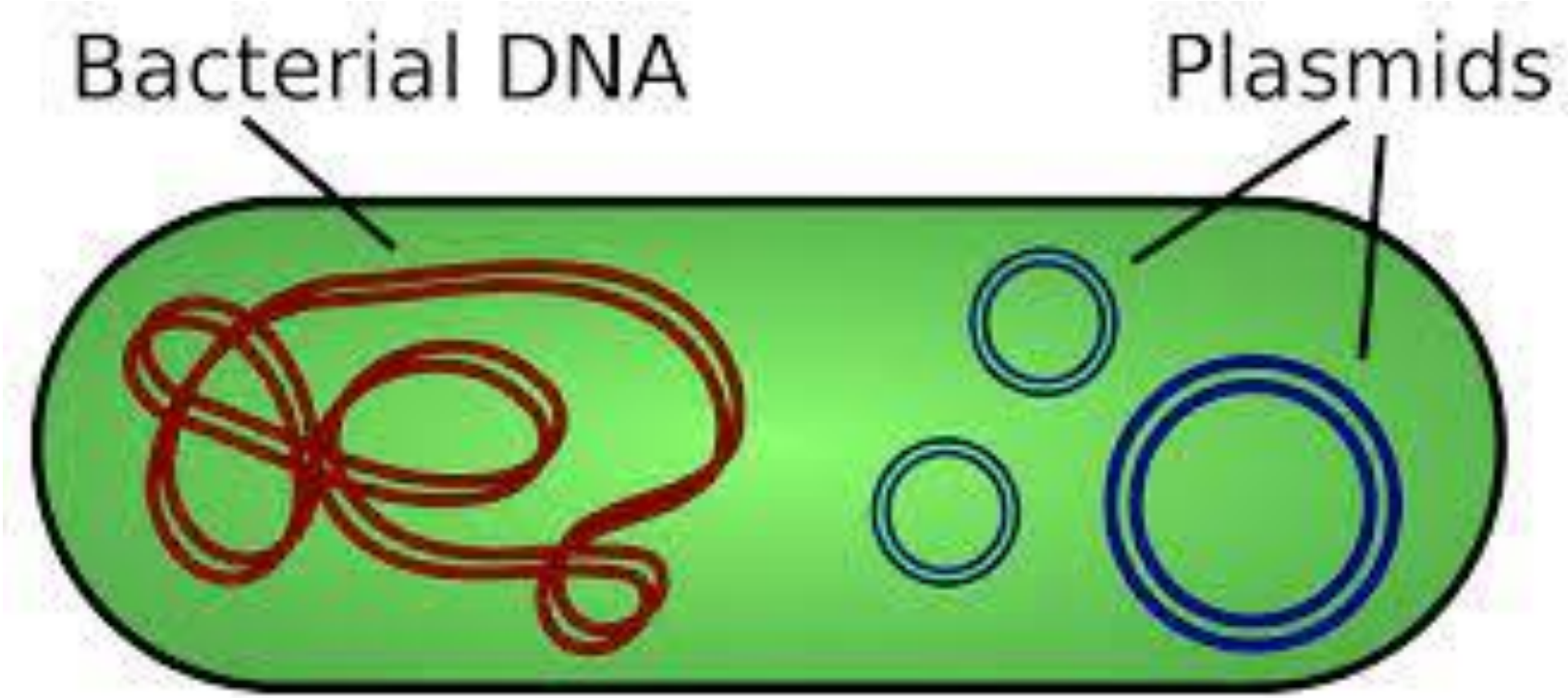
Actinomycetes share the following physicochemical properties::

1.Actinomycetes usually have **1-2 µm** diameter.
2.They generally possess a rod shape with a filamentous or branched structure. The filaments contain **mumaric acid**.
3.Most of the species are **aerobic**, while a few are anaerobes to facultative aerobes.
4.Cell wall and internal structures are similar to bacteria. The cell wall of actinomycetes consists of **mycolic acid**.
5.The growth or reproduction of actinomycetes is slower than the bacteria and fungi. Hence, actinomycetes are sometimes called as "**Slow growers**".
6.They are having **60-78%** of G+C content.
7.Actinomycetes are most abundant in **soil** (10^6-10^8g) and **marine habitat**.
8.The majority of species are usually non-motile, non-capsulated and non-acid fast.
9.They show optimum growth at **alkaline pH**.

1.Germination: The spores of actinomycetes remain dispersed in the environment as a "**Free spore**". Free spore remains dormant until the stage of germination. When the spores get favourable conditions, they start the germination process by forming a germ tube.
2.Vegetative growth: The germ tube promotes vegetative growth, which eventually gives rise to the substrate and aerial hyphae. First, a germ tube will produce a primary mycelium, i.e. **substrate hyphae** that grow within the media. After the growth of primary mycelium, a secondary mycelium, i.e. **aerial hyphae** forms above the substratum.
3.Coiling: During unfavourable conditions, the aerial hyphae turn into a **spiral shape**.
4.Septation: At this stage, a **septum** forms between the vegetative hyphae.
5.Spore maturation: A septum within the vegetative hyphae maturates and forms a **chain of spores**. Thus, the spores originate via fragmentation or swelling of the hyphae.
6.Release of spore: During unfavourable conditions, the spores detach from the vegetative hyphae and remain free in the environment.

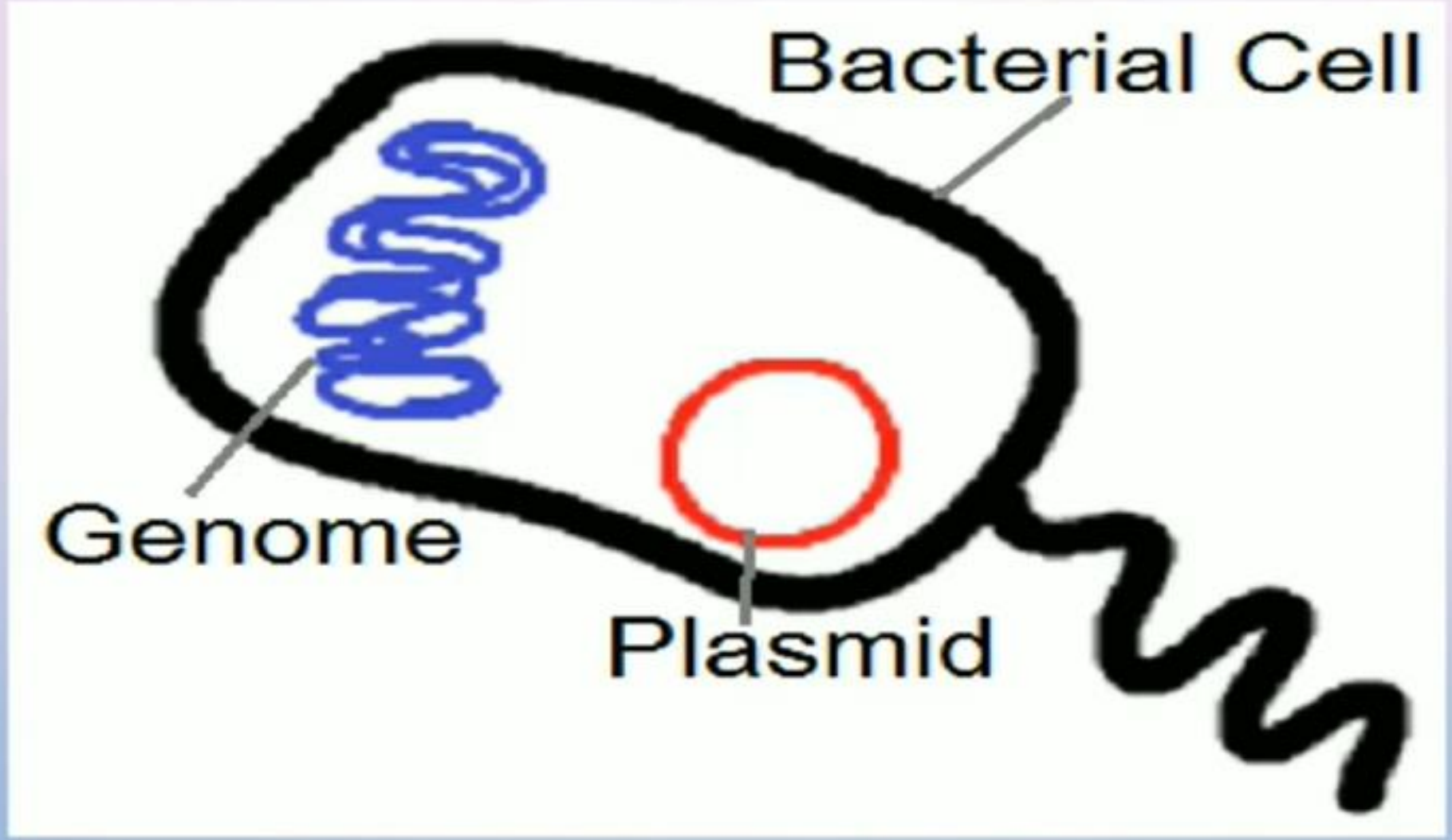


Plasmid



INTRODUCTION

- Plasmids are extra chromosomal, small, circular, supercoiled, double stranded DNA molecule present in mostly all species of bacteria.
- The term plasmid was first introduced by Joshua Laderberg in 1952.
- They are distinct from the normal bacterial genome and non-essential for cell survival.
- Plasmids are also known as
  sex factors
  conjugans
  extra-chromosomal replicons and
  transfer factors.



Present in *Pseudomonas, E. coli, Bacillus, streptococcus, Staphylococcus, Streptomyces* etc.

PROPERTIES OF PLASMID

- Plasmids are extra-chromosomal DNA elements.
- Replicate independently.
- Contain genes that are non-essential but often beneficial to the bacterium.
- Plasmid size varies from 1 to over 1000 kbp.
- Absence of plasmids doesn't kill bacterium, but their presence provides additional benefits to the bacterial cell.
- Certain plasmids are known to be linear. Eg- ***Borrelia burgdorferi***

PROPERTIES OF PLASMID

- **Copy number** – it refers to the number of copies of a plasmid present in a cell. Larger plasmids are present in smaller numbers and small plasmids may be found in high copy numbers .

- **Compatibility**– it refers to the ability of two different plasmids to coexist stably in the same bacterial cell.

GENES OF PLASMID

1. Plasmid contains both *Tra* gene and Ori T sites.

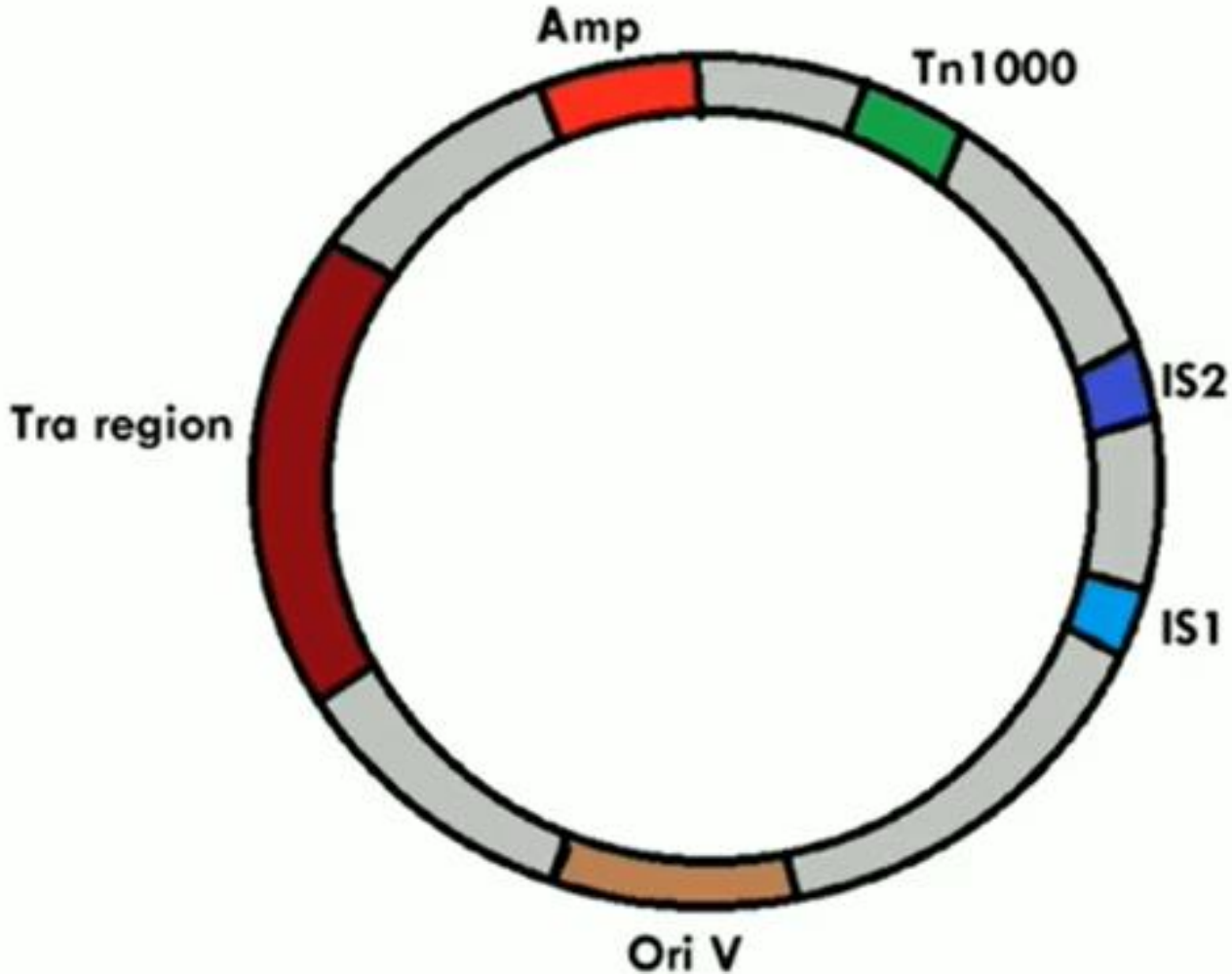
2. The F factor is self mobilizable, it can transfer itself to other calls. This transfer initiates at ori T site.

3. A plasmid must contain an origin of replication (*ori V*).

4. The F factor also contains insertion sequences (IS2, IS3 & IS3b) and the transposon Tn1000.

5. Relaxase- it is a specific endonuclease which act on plasmid at oriT site.

PLASMID



PLASMID CAN INTEGRATE INTO CHROMOSOME THROUGH 2 MECHANISM.

Recombination: plasmid can recombine with chromosome when plasmid and chromosome share common sequences (homologous sequences)

Transposition: plasmid can insert itself into chromosome by transposons and results in formation of Hfr.

TYPE OF PLASMID

1. CONJUGATIVE PLASMID : PRESENCE OF TRA (TRANSFER) GENES

a) **Conjugative plasmids** - contain *tra* genes, which perform the complex process of conjugation, the transfer of plasmids to another bacterium.
e.g., F plasmid, many R plasmid & some Col plasmid.

2. NON-CONJUGATIVE PLASMID : ABSENCE OF TRA GENES.

b) **Non-conjugative plasmids** - incapable of initiating conjugation, hence they can be transferred only with the assistance of conjugative plasmids.
e. g., many R plasmid & most Col plsmid.

THE PLASMIDS CAN ALSO BE CATEGORIZED ON THE BASIS OF THEIR FUNCTIONAL PROPERTIES. THERE ARE SIX MAIN CLASSES.

1.F PLASMIDS OR FERTILITY PLASMIDS

2.R PLASMIDS OR RESISTANCE PLASMIDS

3.COL PLASMIDS

4.DEGRADATIVE PLASMIDS

5.VIRULANCE PLASMIDS

6.CRYPTIC PLASMIDS

F-PLASMID: F plasmids are conjugative plasmids which contain tra genes.
In addition to tra genes, it contains a number of other genetic sequences responsible for incompatibility, replication & other functions.

R PLASMIDS: Plasmids that carry & transmit antibiotic resistance genes from one bacterium to another are known as resistance (R) plasmids or resistance factors.
These plasmids provide protection on to bacteria both from human medicine & from antibiotics produced naturally in the soil. This plasmids are large size & present in low copies per host cell.

COL PLASMID: These plasmids synthesize proteins – **colicins**.
They are also known as **bacteriocinogenic** plasmids because they produce **bacteriocins**.
This protein can kill closely related bacteria that lack a col plasmid of same type.
In most cases, colicins are inactive against a cell that contains a related col plasmid.

DEGRADATIVE PLASMID: These plasmids are the group of plasmids which enable the digestion of unusual substances, e.g.,toluene, xylene, napthalene, camphor, chlorobenzoate, salicylic acid etc.
These plasmids provide genes that allow bacteria to grow by breaking down various unusual metabolites or industrial chemicals including herbicides.
For example, TOL plasmids that contain tod genes which encode enzymes that catalyze degradation of toluene

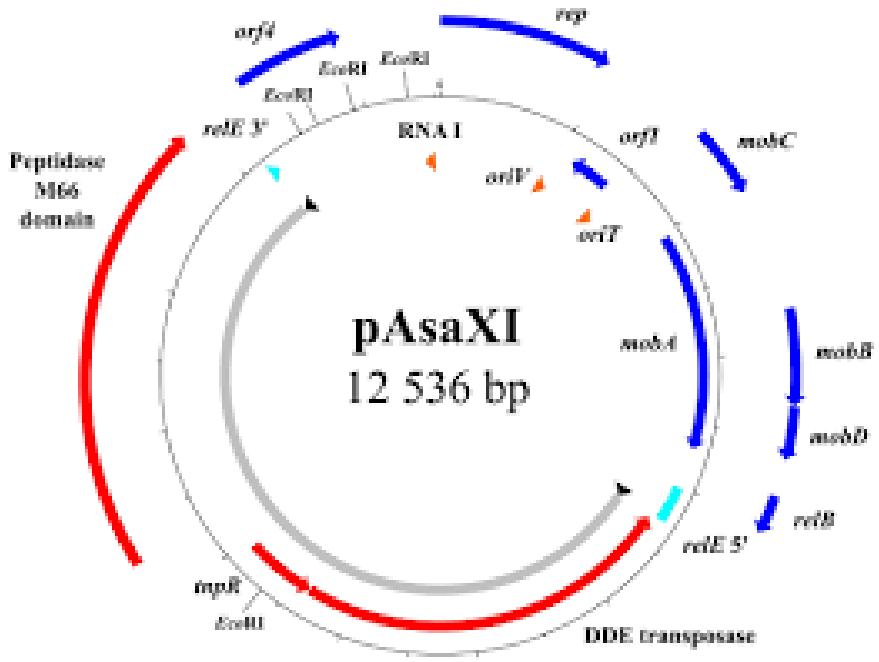
VIRULENCE PLASMID: Plasmids carry genes for various characters which help bacteria to infect higher organisms both plants & animals, including humans. Bacteria having Virulence plasmids are able to attack animal cells by attaching to their cellular membrane & release toxins. An example of Virulence plasmids is tumor-inducing plasmid(pTi) of A. tumefaciens that causes crown gall tumor on wounded plant cell.

What is a cryptic plasmid?

A cryptic plasmid is one that has no genes which benefit its host organism.

Cryptic plasmids are essentially in a commensal relationship with its host - the plasmid benefits by utilizing its reproductive machinery, but the host gains no benefit.

A cryptic plasmid can be removed from its host without any ill effect to the host.



Type	Example
Degradative plasmid	TOL plasmid of *Pseudomonas putida*
Virulence plasmid	Ti and Ri plasmid
Fertility (F) plasmid	F plasmid of *E.coli*
Resistance plasmid	pRP4 of Pseudomonas spp
Col plasmid	ColE1
Cryptic plasmid	pAsaXI

Ti Plasmid

The Tumour inducing or Ti plasmid is present in the bacterium *Agrobacterium tumifaciens*. It is widely used now as a cloning vector to deliver desirable genes to the host plant to get **transgenic plants.** The main characteristics of Ti plasmid are:

•Size of the plasmid is ~ 250kbp

•There are different kinds of Ti plasmids based on the different genes they possess, which code for different opines, e.g. leucinopine, nopaline, octopine, etc.

•It is a pathogenic species to many dicotyledonous plants. It causes crown gall disease in plants.

•It contains one or more T-DNA region

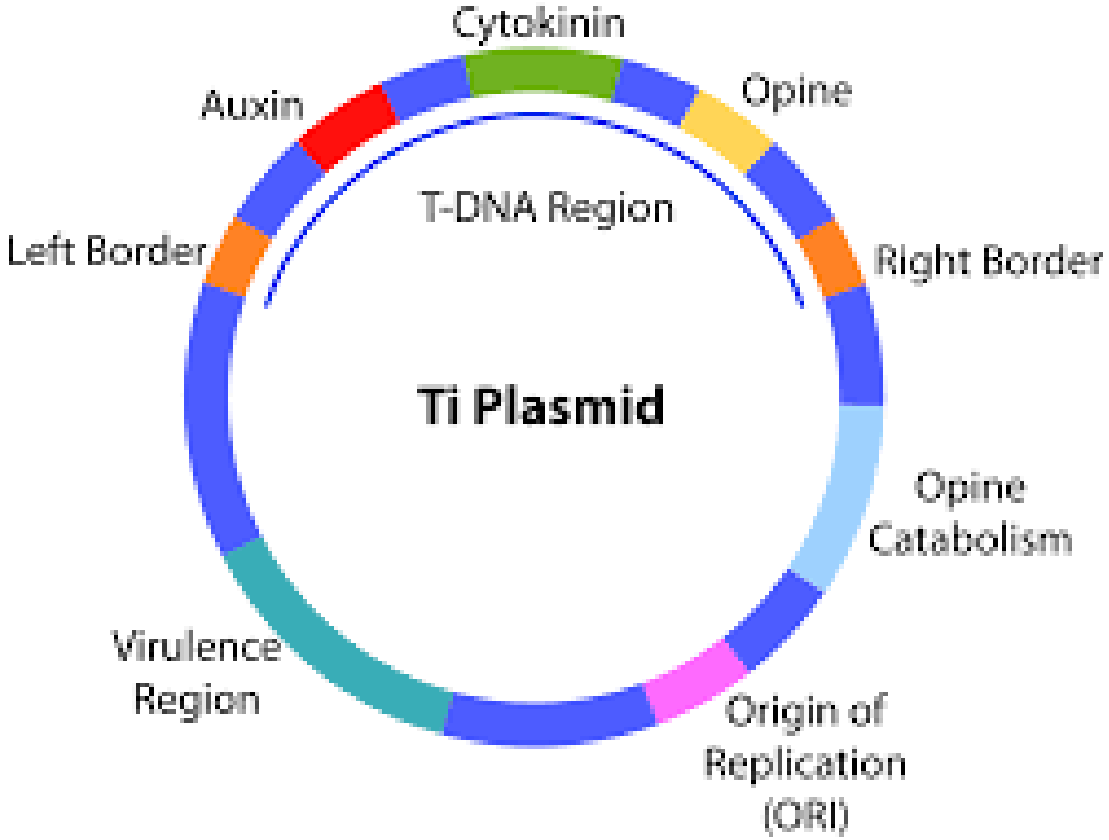
•*Agrobacterium tumifaciens* has the ability to transform the normal cells into tumour cells by inserting a DNA piece known as T DNA and it starts producing chemicals, that are required by the bacterium

•After inserting the desired gene into Ti plasmid, it loses its pathogenic ability but is still able to insert the desired gene into the plant cell

•It contains *vir* or virulence genes**,** which transfer T-DNA region to plant cells and gets integrated into the plant genome

•Ti plasmid can be modified as per the requirement to insert the desired genes

•*Agrobacterium tumifaciens* is called “nature’s genetic engineer”


Cytokinin
Auxin
Opine
T-DNA Region
Left Border
Right Border
Ti Plasmid
Opine
Catabolism
Virulence
Region
Origin of
Replication
(ORI)

Characteristics of Naturally-occurring Plasmids

1. They influence characteristics of their bacterial host
2. They replicate independently of the main chromosome.
3. They are usually specific to one or a few species of bacteria.
4. They can undergo reversible integration into bacterial chromosome.
5. A few plasmids can pick up and transfer chromosomal genes.
6. They can be transferred by conjugation.
7. They usually contain upto 40 genes.
8. They do not occur free in nature. It is important to note that except for the last three properties, the other characters are shared with viruses.

THANK YOU