Morphological study of bacteria:

The important step to indicate of bacteria is, the morphology by:

1. Un stained preparation (wet preparation):
   Take drop of bacteria culture and put it on the slide and covered by covering slid. The disadvantage of this method is the presence of grayish through medium.
2. Stain preparation:

   It is mostly used for bacterial study; we take dry fixed film by take drop of bacterial culture on glass slide allow to dry in air , then make fixation by dry heating three time or ovoid charring the slide and then stain by two main method:

   1- Grams stain.

   2- Ziehl-Neelson.

Grams stain:

This discovered by Hans Christian Gram in 1884, the importance of this stain by divide the all bacteria into:

a. Gram Positive +ve.

b. Gram Negative – ve.

Ingredient of Gram stain:

It composed of four ingredients;

1. Basic stain -------Violet.
2. Mordant ---------- مثبت للصبغة.
3. Decoulourization مزيل للصبغة.
4. Counter stain الصبغة البديلة. Red or pink.

1. Basic stain :
   It is injection of crystal violet in 0.5%.
2. Mordant :

It is the Grams iodine when consists of iodine 1 gm potassium ioded 2g + water 100 gm.

3. Decolourizer:
   Absolute ethyl alcohol 95%.
4. Counter stain:
5. It is safranine which composed of 2.5 % alcoholic solution and 0.5 % water.

Slide stain:

1. Apply crystal violet stain for 30 second.
2. Wash with water.
3. Apply the Gram iodine solution for 30 second.

4. Wash with water. At this step all bacteria can be stain with violet color.
5. Apply decolourizer for 30 seconds.
6. Wash with water .After this step group of bacteria appears unstained.
7. Apply counter stained for 30 second.
8. Wash with water.
9. Klot نشف the slid, dry and examine. After this step the unstain group stain with red color.

The red color bacteria called Gram – ve bacteria. This due to the mechanism of Gram –ve reactive which are:

1- Gram +ve are acidity and have affinity to basic stain.

2- Gram –Ve bacteria the cell wall of them is impermeable to alcoholic solution that the alcohol not penetrates the cells and remains the stain.

3- Differences of chemical composition of the cell wall in Gram – Ve bacteria for example type of bacteria have more fat in cell wall so that the alcohol dissolved the fat and the decolourizer penetrate the cell and remove the stain.

4- Present of specific material in bacterial cells such as magnesium consist with crystal violet and I_2 insoluble complex in the alcohol that there is no decolorized.

These are the four theories of the mechanism of Gram reactive. The improve البرهان of the second and third theories take bacterial cell e cell wall digested and the G+Ve bacteria change to G-Ve bacteria.

The improved of the fourth theories by take bacterial cell and react with bile salts or ribonucleal enzyme which digested Mg so the Mg is disappear and the cells change from G+Ve to G-Ve.

Notes on G. Stain:

1. Some G+Ve bacteria weekly (+Ve),It means easily decolorized like Coryne bacterium which you increase the alcohol it will converted to (-Ve) and take G-Ve stain.
2. G+Ve is characterized of living or young cells 24-48 hours.

Morphological study of film:

1. Size of the bacteria:

This size assist to know the types of bacteria, the measurement Unit is micrometer (micron) (Mu).

I Mu micron = 1/1000 m.m.

1 nanometer (millimicron) nm = 1/1000 micrometer (used for viruses).

1 angstrom = 1/10 nm (A^o)

Smallest size that eye can see is(100) micron, all bacterial are less than (100) micron, so we must use ordinary microscope, in this microscope we can see bacteria of length(0.2 um)as a minimum have size less than (0.2 um).

Bacillus have length and Brith صورة 0.5x2.3 um

Some bacteria have diameter صورة

RBC = 7 um diameter

Coci = 1 um

Influenza bacillus $0.3x\ 7^{-1}$um.

Typhoid bacillus 0.5×3^{-8} .

Anthrax bacillus 1×3^{-8} .

1- Shape and arrangement of bacteria:

shape	Cocci	Bacilli	Coma or curve	Spiral	Filamentous
	Cluster	Single	Single form	Wave length	Branch
	Diplo	Diplo		Borretella	Non branch
	Tetrads	Angular		Trepanema	
	Sarcivia	Chaines letter		Leptospira	
	Chain form	Spor forming Dram stick			

Bacterial physiology and Metabolism:

Chemical composition of bacteria:

The basic chemical composition of bacteria is essentially similar .The major componance of bacteria is water which make up about 80% from bacterial cell and 20% dry matter which is organic matter and inorganic matter (ash).

Organic matter------ Cho, fat, protein, amino acid and organic acid.

Inorganic matter------- Nacl, inorganic acid.

Organic and inorganic element in microorganism given in % of dry matter (dry weight) which is 20%.

Element	Prokaryotes	Eukaryotes
Organic		
Nucleic acid	10%	5%
Proteins	40%	45%
Polysaccharides	15%	15%
Mucopeptides	10%	0%
Lipids	15%	25%
Inorganic(ash)	10%	10%

Cholesterol:

Cholesterol is unit prosperity of eukaryotes. These percentages are the every of the components and can be increase in the comfortable medium and decrease in uncomfortable conditions (medium). 50% of dry matter is protein (Nucleic acid + proteins) nucleic acid (DNA, RNA) regulate the living process like nutrition and reproduction.

Physiology and metabolism of bacteria:

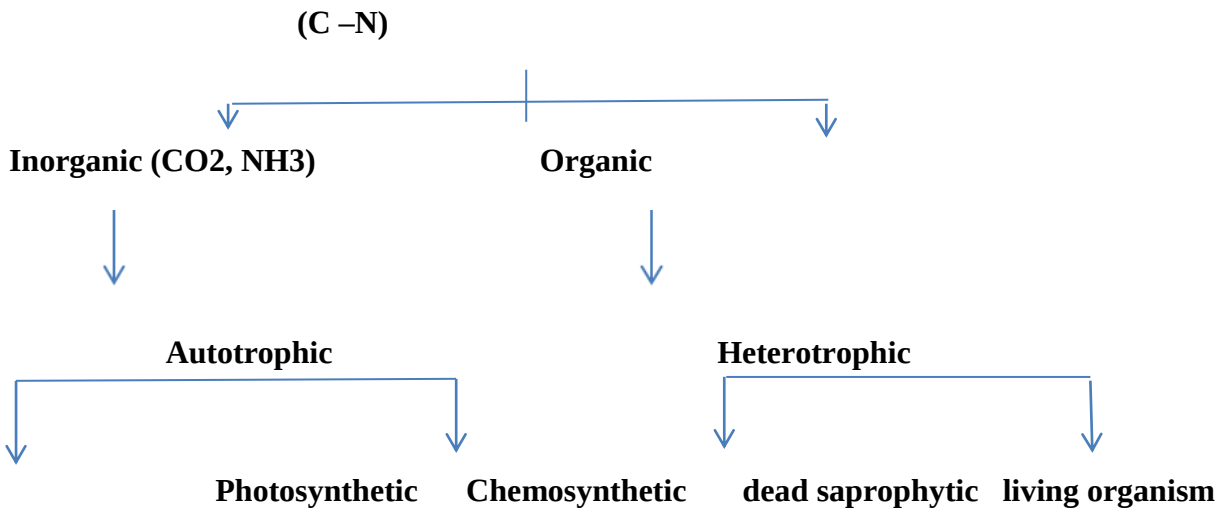
1. Nutrition and growth requirement.
2. Action of bacteria on substrates (several nutritional substances).
3. Bacterial enzyme.
4. Metabolic products (pigments and toxic).
5. Reproduction and growth curve.

Nutrition and growth requirement:

It means that bacteria need inorganic material and the pathogenic bacteria need organic material or complex nutrition .The most element needed by bacteria are ;C,N, H_2, vit., mineral ,the importance element is C ,N.

Grlar Jensen in 1909 distinguished two type of bacteria according to their requiring to C and N:

1. Bacterial need organic material to contain C and N.
2. Bacterial need inorganic material to contain C and N.



Essential nutrition and growth requirements:

The substance which needed by bacteria are;

1. Carbon put Carbone in culture.
2. Nitrogen (peptone or amino acid put in culture).
3. Growth factor vit.B, Co enzyme. Coenzymes join with bacterial enzyme to activity the digestion.
   Staphylococcus --------- vit. B1
   Streptococcus --------- vit.B 2
   Lacto bacillus --------- vit. E and B12
4. Mineral K, Na, CL, .The most important substance of mineral is sulpher, phosphate also ferrous and magnesium need for activated bacterial enzyme.
5. Blood
   Gonococci need blood in its culture to growth.
6. Cases requirement the important gases are O_2 and CO_2.

Bacterial classified according to the living in present or absent of O_2 are:

1. Obligatory aerobic bacteria:

It means bacteria which can live only in present of O_2 e. x. Mycobacterium tuberculosis.

2. Facultative anaerobic bacteria:

Bacterial which growth well in both presence and absence of O2 e.x. Typhoid salmonella.

3. Obligatory anaerobic bacteria:

Bacteria which cannot growth in present of O_2 Clostridium tetani.

The aerobic bacteria contain catalase enzyme ,due to the metabolism of bacteria cells ,the H_2O_2 into H_2O and O_2 while in anaerobic ,there is no catalase enzyme so H_2O_2 is accumulate and causes poisoning to bacterial cells, so it not need the O_2 .

Catalase ⇌ H_2O_2 (hydrogen peroxide) H202

H_2O_2 + O

4. **Microcerophilic :**
   Bacteria which can growth in small amount of O_2 e.x. coryne acne مسببات حب الشباب.

7. CO2:

CO_2 is essential for growth of all bacteria except group of bacteria need highly percentage

Of CO_2 such as brucella abortus this group need to present of CO_2 10%.

8. Moisture:

It need for saturation of bacterial cell e: x gonococci growth in higher moisture while TB need low moisture.

9 .**Temperatures:**

The temperature in which the bacterial cell have the best growth in it called optimum T. of most pathogenic bacteria is $37C^0$ minimum T. it is the lowest T. which bacteria can live in it 43 C^0 .
According to temperature there are three groups of bacteria:

1- Psychrophilic(water bacteria):
Bacteria which need lower T.(most are nonpathogenic).
2- Mesophilic :(pathogenic bacteria):
Which need T. more than psychrophilic 37^o.
3- Thermophilic (solid and milk bacteria):

10. PH(Hydrogen ion concentration) :

Acid (2,3,4, 5, 6) 7 pH ⟵ (8, ~~9, 10, 11) alk~~aline

Bacterial prefer to neutral or slightly alkaline pH this means from (Optimum 7.2 – 7.6 pH) .some bacteria have affinity to acidity which called acidophilic e.x lactobacilli pH 6-6.2. , some bacteria prefer to alkaline media such as vibrio cholera pH 8.6.

Action of bacteria on different substrate

1. **The action of bacteria on carbohydrate:**

Bacteria attached one or more of the sugar, the digestion of CHO by carbohydrates enzyme .Carbohydrolyses either polysacchrideses e:x: starch ,glycogen or glycosidase e:x: disaccharides or the monosaccharide.

$C_6H_{12}O_6 + H_2O$ Lactic acid → acetic acid

$CH_3CHOHCOOH + CH_3COOH + C_2H_5COOH + CO_2$

Ethyl alcohol $+ H_2$

The fermentation of CHO by bacteria in the nature called fermentation of CHO.

a. Action of bacteria on protein:

Proteolytic activity means digestion of bacteria protein large group of bacteria can able to digest the protein. Proteinase enzyme is effect on protein and converted it to polypeptide and this by polypeptidase enzyme convert to amino acid. Proteolytic activity assists to Know the type of bacteria because some bacteria digestion of gelatin and other not get anaerobic bacteria have need proteolytic enzyme.

b. **Action of bacteria on fat:**

Lipolytic activity bacteria make digestion to fat by enzyme lipase and converted lipid to fatty acid and glycerol e: x Mycobacterium Tuberculosis.

2- Enzyme of bacteria:

There is about 110 enzymes in each bacteria enzyme means organic catalase, accelerate the chemical reaction .Bacteria enzyme s are thermolabile effect by temperature, PH and moisture. There are two type of bacterial enzyme.

a. Extracellular enzymes.
b. Intracellular enzyme.

Extracellular enzyme means that it is secretes outside to digested the surround substance. The extracellular enzyme are carbohydrates ,proteinases, lipases, after the digestion of substrate ,these digestion substance inter the cells by permeases, in side of cell it attacked to intracellular enzyme to convert the substance in to end products .This enzyme called energy transformation enzymes.

Adaptive enzyme:

Enzyme produces by bacteria with long time on special substance.

البكتريا تعمل على تكييف نفسها لإفراز أنزيم على مادة خاصة البكتريا

Substrate: it is the matter effect by the enzyme.

3- Bacteria Metabolic Product:

Pigment and toxin metabolic is the importance of metabolic product.

1. Pigments :

Bacteria can give many color pigment, there are two type of pigment.

a. Endopigments :

Pigment secreted inside the bacterial cell, it called nondiffusable due to non out of the cell. E:x: Staph aureus which make orange pigment.

Staph citrus which make yellow pigment.
Staph ortia marseen which make red pigment.

b. Exopigment :

Pigment secreted outside the bacterial cell, it is diffusible pigment e. x:
Pseudomonas argenosa which give pyocyanin pigment has bluish green in color.

Factors influence pigment production:

Pigment production depended on certain media, in addition

1. Of CHO, glycerol, milk assist the production of pigment produce.
2. Temperature of $25C^0$;
   By 25^o of culture and Temp.

Toxic production by bacteria:

It has not been known the certain bacteria produce toxins responsible for the disease .The virulence of bacteria and pathogenic depended on :

1. Invasiveness :قوة غزو البكتريا
   The capacities of bacteria to infect the tissue of host .bacteria secrete aggressive substance.
2. Toxigenicity :
   Ability of bacteria to produce the power of toxin. There are two type of toxins:

A. <u>Endotoxins or intracellular toxin:</u>

They are not diffuse outside the cells until the death or degeneration of cell .It is intracellular production.

Preparation of endotoxin:

1. By natural autolysis in which we leave the bacteria to die and then the toxin pass out of cell.
2. Preparation by freezing and thawing ;in which the freezing of bacteria take place and then the rapture is occur so we put bacteria in water to get toxin from it.
3. Heating; we heat the bacteria in favorable temperature over$100C^0$ .The detoxication possible is occur, so the heat stable is about $60\text{-}80C^0$.

B. <u>Exotoxin or extracellular</u>

Toxins produce and diffuse outside the bacteria this toxin not in bacteria cells.

Preparation of exotoxins:

1. Centrifugation.
2. Filtrations.

After make the cultivate and make centrifuge so the bacteria precipitate in the bottom while in filtration the toxins fill down and bacteria is attach the filter paper.

Prosperity and deference between exotoxins and endotoxins bacteria

	Property	Exotoxin	Endotoxin
1	Diffusability	Diffuse	Not diffuse
2	Heat 60-80C^0	destroyed	Not destroyed
3	Toxigenicity	Highly toxic	Low toxic
4	Specificity	Specific	Not specific
5	Nature	Protein nature	Phospholipid, polysaccharides
6	Production	G+ bacteria	G- bacteria
7	Action of formalin	Detoxication Formal toxoid	Not effect
8	Antigenicity	High	Low

Leucocidin is specified to affect RBC.
Hemolysin is specified to affect WBC.
Exotoxin + formalin ⟶ formal toxoid (not toxic substance

Heat labile:
Instability when exposure to heat.
Antigenicity:
Capacity to produce toxin.

Examples of bacteria produce exotoxins

1. **Diphtheria:**
   The dangerous of diphtheria bacteria in the toxin not in the microorganism.

Tetanospasmin:
It is exotoxin substance which produce by CL.tetani and get spasm of the muscle contraction

2. **CL.tetani:**

   Also it is highly exotoxin and gets tetanus disease.

3. **Staphylococci:**

   Also it is highly exotoxin.

Examples of bacteria produce endotoxins

1. **Salmonella typhoid:**
   It is endotoxin bacteria.

Agressin:

Substances produced by certain bacteria species to assist the organism to infect and multiply in the tissue e.x. invasiveness, B-anthrax. The agressin make inhibition to the first mechanism of the body. So it is different from toxin.

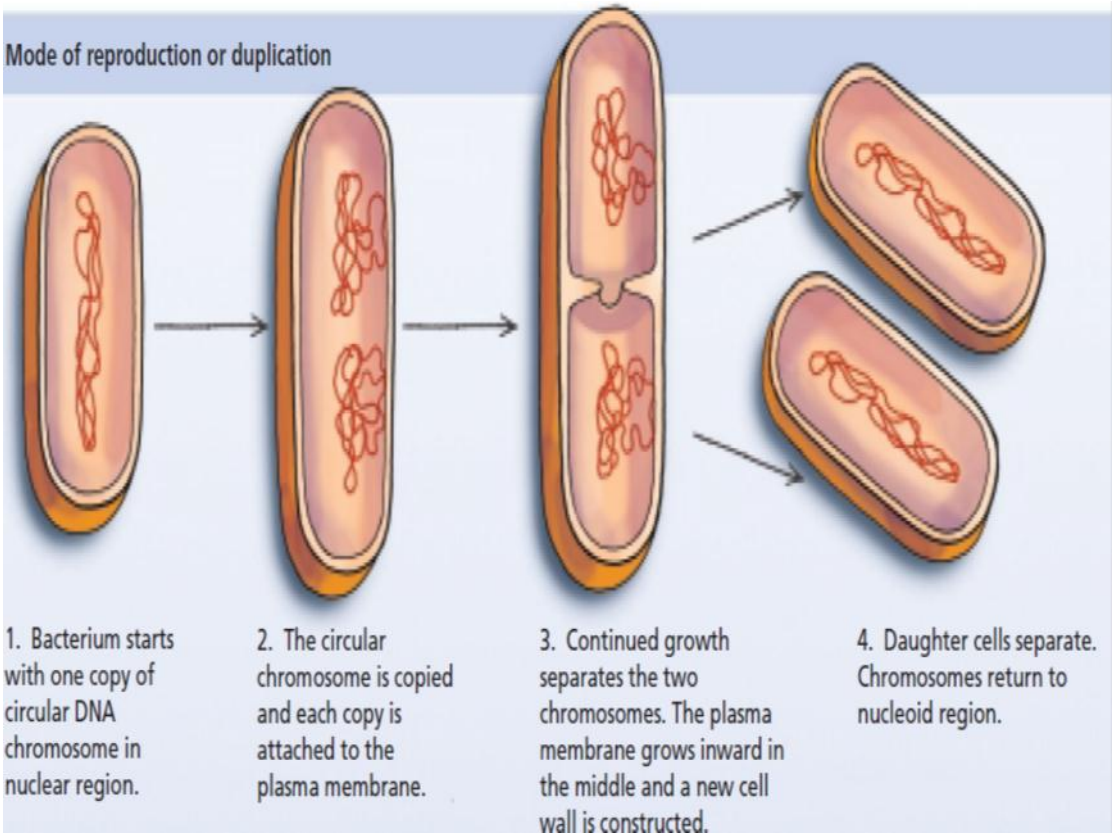
Reproduction and growth of bacteria

A bacterium is reproduction by simple binary fission, this process very rapid, it occurs in each 20 minute.

Generation time:

It is the time between the growth and the complete separation of the two individual. In one day the simple bacteria give many bacteria about 10^{12} or. In the culture media the bacterial growth the bacterial growth is low due to:

1. Less of nutrient substances.
2. accumulations.



Growth culture:

When seeding Bactria culture and put in suitable temperature, feeding, it grows up and multiply in consistent.

Logarithmic phase:

It is the step of bacterial growth.

1- Lag phases:

Bactria attached itself to the new media in this phase we see

a. No multiplication

b. Increasing in size without, cell division.

c. Time of this phase (duration) is varying from a few hours to some days this variety depends on:

1. Nature of organism ex, E.coli 1-4 hours, T.b 3-7 day.
2. The more suitable media the shorter lag phase.
3. Stage from which the organism was came.

2. Logarithmic phase or exponential phase or phase of increasing

a. In this phase gets division of maximum speed, geometric increasing in the number of Bacteria cells.

b. The duration is varying in different bacteria; it's about 6-8 hours.

3. Stationary phase

It means the constant number of bacteria cell in this phase

1- The bacterium is ceased (يقل -يتوقف) to multiply at maximum rate.

2- Death rate began

The bacteria cells which produce from the division is equal to death cells in this phase 4.

4. **Decline phase**

Phase of decreasing, in this phase

1- No division or the division is slowly rate.

2- Increase in the death rate.

So there are no bacteria no living bacteria in a few days.

How to indicate the growthing of bacteria, by:

1- Forming the colony, the colony is due to the multiplication and the increasing in the number of bacteria and aggregation is occurring. The number of bacteria in the media is about 10^9 .

2- In the fluid media the appearance of turbidity makes the indication to fluid media

The Bacterial Chromosome:

- Most bacterial chromosomes are circular
- Many have been fully sequenced
- Many genes have been identified and mapped using gene transfer techniques such as conjugation, transduction, and transformation

Bacterial genetics

The genetic constituent is control by nucleoli, the nuclear body consist of thread composed of DNA constituent of chromosome which carriers gene, the chromosomes with gene called genome , so the genome means chromosome carried a gene .

The chromosome divides into two couples one of these couples goes to the new cell and the other remain in the first cell, third process is called replication.

In some bacterial frequency from 1/1000, chromosome divides to middle and the other part does not have a division this leads to a mutation, so the mutation means alteration change constituent in the genome dues to a replication mistake.

There are two methods that the mutation mistakes take a place which are:

Mechanism of gene transfer in bacteria: Gene transfer either

- **Transformation**

- **Transduction**

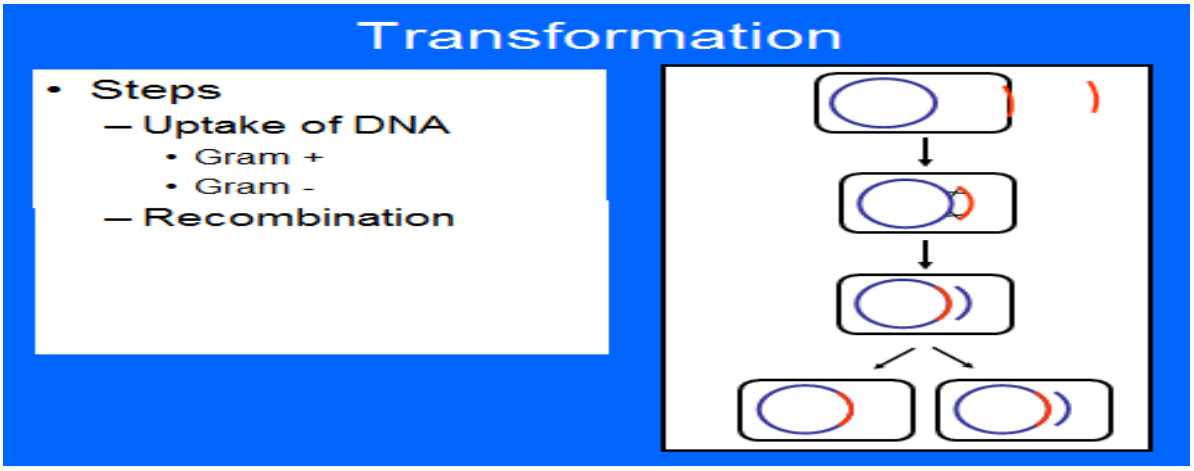
- **Conjugation**

Transformation:

There is a transfer of DNS from a bacterial cell to another.

- Types of transduction

1. Generalized Transduction : in which potentially any dornor bacterial gene can be transferred.
2. Specialized Transduction : in which only certain donor genes can be transferred



Conjugation:

- Definition: Gene transfer from a donor to a recipient by direct physical contact between cells

- Mating types in bacteria

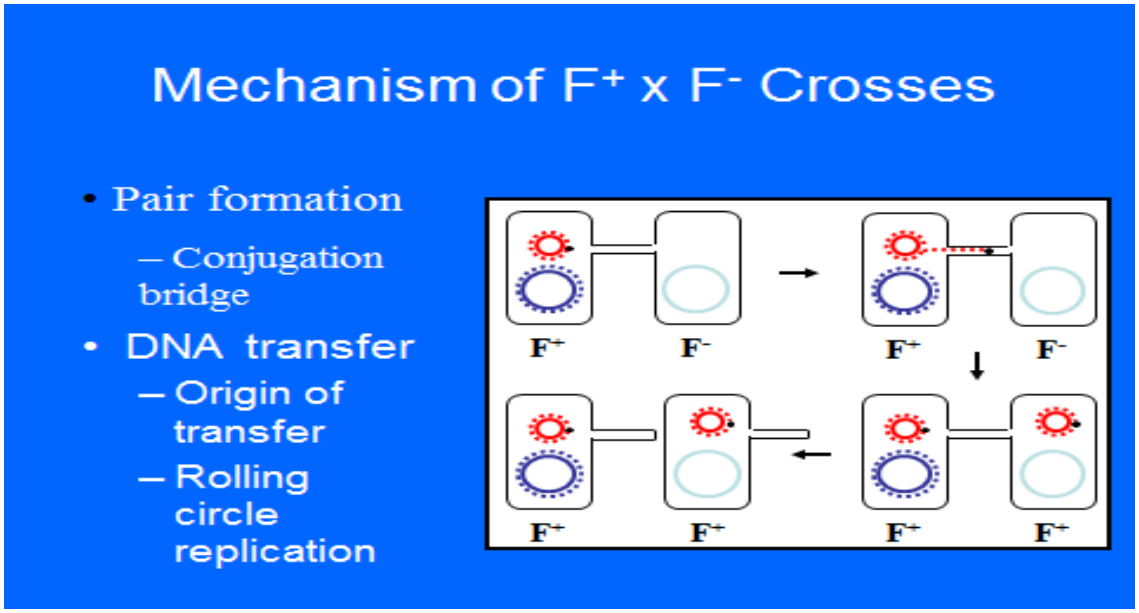
– Donor

• F factor (Fertility factor)

– F (sex) pilus

Recipient

Lacks an F factor



Transduction:

There is a an alteration dues to the bacterial cell requiring the transferring DNS from surrounding media , free DNA produces by lysis of some bacteria after it take DNA and makes a correction in it is genome.

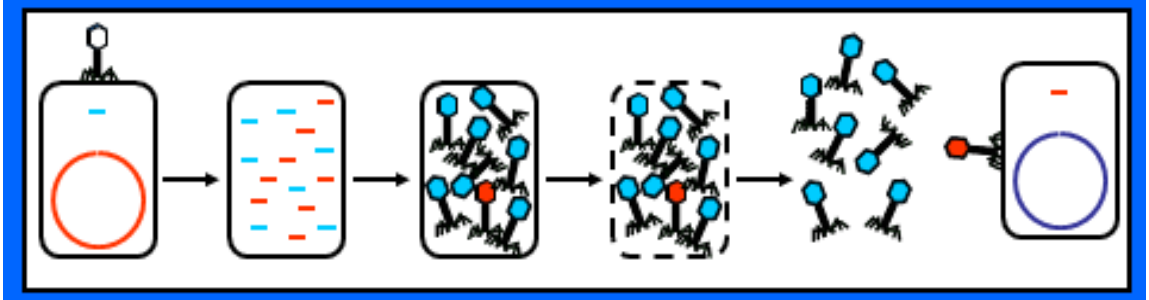
Pneumonia coccus type 1 Smooth

Pneumonia coccus type I I Rough

Make a mixture of living pneumonia coccus type 1 and Pneumonia coccus type I I and inject it intraperitoneum in a guinea pig, he saw the pig is died , so he thought that the infect occurred by type 1 while in the true the genetic characteristic transported from type I I to type 1 and got the infect .

Generalized Transduction

- Infection of Donor
- Phage replication and degradation of host DNA
- Assembly of phages particles
- Release of phage
- Infection of recipient
- Homologous recombination

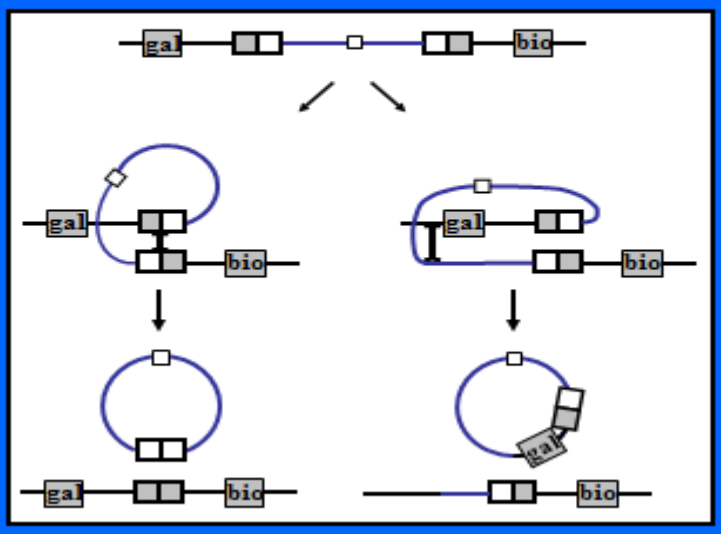


Potentially any donor gene can be transferred

Specialized Transduction

Lysogenic Phage

- Excision of the prophage
- Replication and release of phage
- Infection of the recipient
- Lysogenization of the recipient
  - Homologous recombination also possible

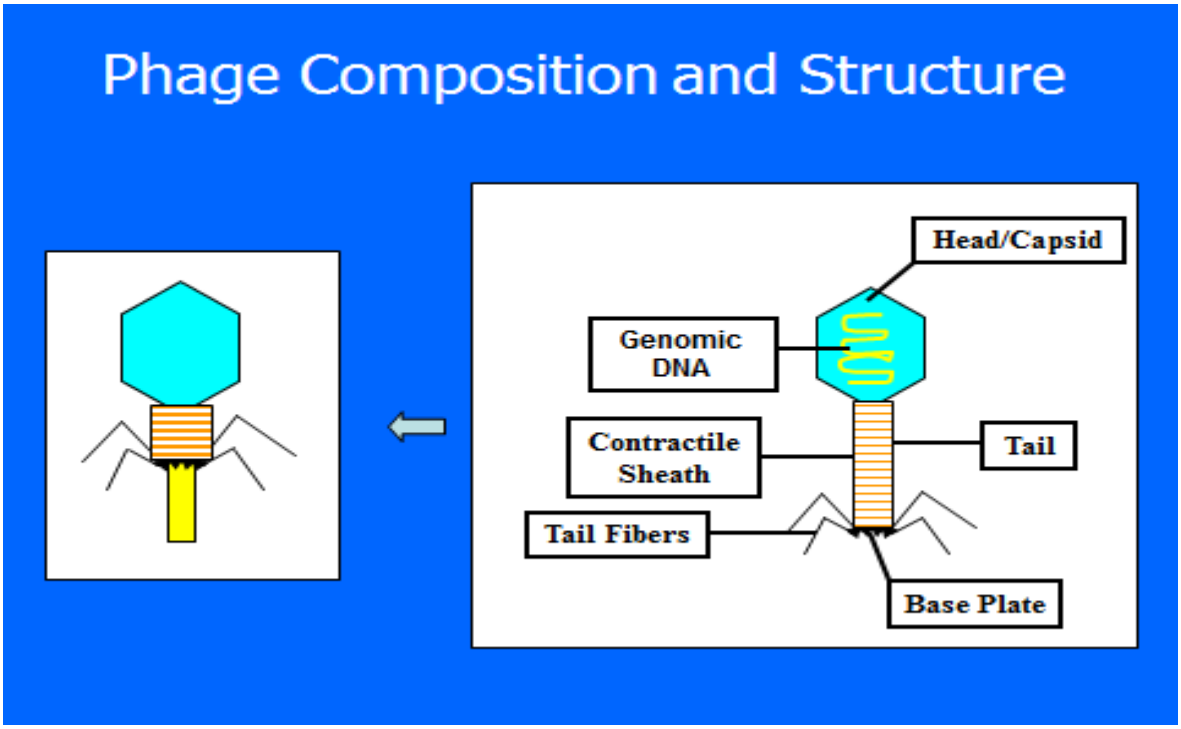


6. Bacteriophages (bacterial viruses):

- virulent

- temparate:

lysogey



Types of Bacteriophage:

Lytic or virulent :

Phage that multiply within the host cell,

lyse the cell and release progeny phage (*e.g.* T4)

Lysogenic or temperate phage:

Phage that can either multiply via the lytic cycle or enter a quiescent state in the bacterial cell. (*e.g.*, λ)

– Expression of most phage genes repressed

– Prophage – Phage DNA in the quiescent state

Lysogen – Bacteria harboring a prophage

Plasmids:

• Definition: Extrachromosomal genetic elements that are capable of autonomous replication (replicon)

• Episome - a plasmid that can integrate into the chromosome

Plasmid- Coded Functions:

• Fertility

• Resistance to:

- antibiotics
- irradiation
- phages

Production of :

- exotoxins
- enterotoxins
- bacteriocins
- Proteases

(cheese)

Metabolism of :

- various sugars
- hydrocarbons

Tumergenesis in

plants

Transducing bacteriophage:

Small organism is attached to bacteria and enters inside of the bacteria and takes a genetic characteristic of it, means the transmission of DNA segments from one cell to another by means of transducing bacteria.

Transducing bacteriophage either remains in the chromosome or separate with the chromosome or it separated localized from the chromosome this called plasmid (drug resistance).

Plasmids (extra chromosomal elements):

- functions
- role in antibiotic resistance (R plasmids)

Drug resistance:

It is a result of extra chromosomal DNA segment.

The mutation results:

There are some indications make improve to genetic variation:

1- S. tarvariation

2- Lose the ability to form a capsule, fimbriae, and flagella.

3- Drug resistance.

4- Virulence of bacteria also changes from an infective bacterium to an uninfected bacterium.

It is possibly to make an artificial mutation by exposure the bacteria to radiations, X ray; those substances are called mutagenic substances.

Transposons
(Transposable Genetic Elements)

- Definition: Segments of DNA that are able to move from one location to another

- Properties

– Inverted terminal repeat sequences (loop formation)

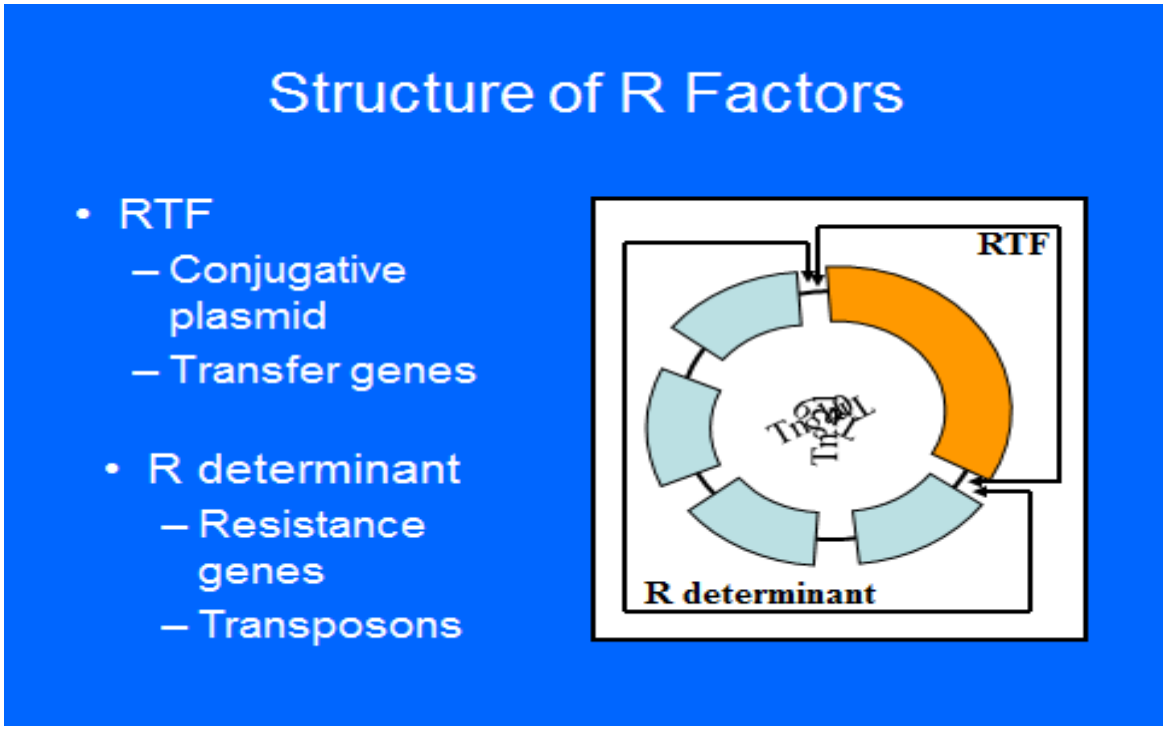
– "Random" movement from one DNA site to another

– Not capable of self replication (not a replicon)

– Transposition mediated by site-specific recombination

- Transposase

Transposition may be accompanied by duplication



Mechanism of Plasmid-Mediated Resistance:

- Production of enzymes for :

- Hydrolysis of β-lactam ring
- phosphorylation
- adenylation
- acetylation
- methylation
- modification of permeability
- other