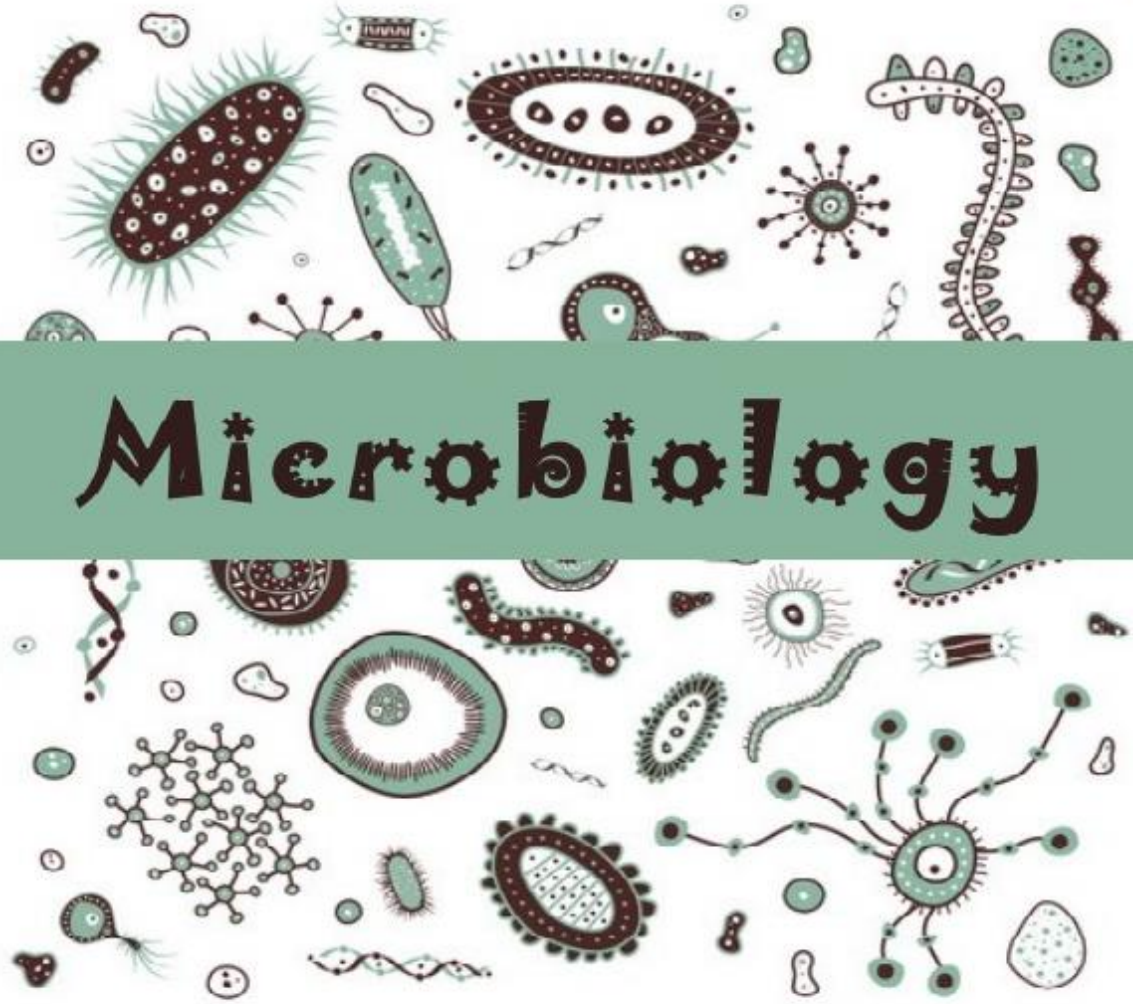


Microbiology

◉ Sheet ○ Slides

Number : 4

Done by : Sally Al.sa'di

Corrected by: Abdulrahman Ibrahim

Subject: Bacterial genetics

Doctor: Asem shehabi

بسم الله الرحمن الرحيم

WHAT YOU HAVE LEARNT FROM THE PREVIOUS LECTURE: (Main points)

- Bacteria are unicellular organisms (prokaryotes) which lack nuclear membrane, nucleolus and other cell organelles like mitochondria, endoplasmic reticulum and Golgi apparatus**.**

-For growth and multiplication of bacteria, the minimal nutritional requirements are water, a source of carbon, a source of nitrogen and some inorganic salts**.**

-Phototrophs are bacteria that derive energy from sunlight while chemotrophs derive theirs from chemical reactions.

- **Autotrophs** are bacteria that synthesise all their organic compounds while heterotrophs are unable to do so.

-**Heterotrophs** depend on preformed organic compounds.

-**Aerobic bacteria** require oxygen for growth and may be obligate aerobes or facultative anaerobes.

-**Obligate aerobes** grow only in the presence of oxygen.

- **Facultative anaerobes** grow in the presence or absence of oxygen.

- **Anaerobic bacteria** grow in the absence of oxygen.

-**Obligate anaerobes** may even die on exposure to oxygen.

- Depending on requirements of temperature for growth, bacteria can be classified as **mesophilic** (25-40ºC), **psychrophilic** (below 20ºC) and **thermophilic** (>60ºC).

-Bacteria divide **by binary fission**.

- The time interval between two cell divisions is **the generation time** or the population doubling time.

- The bacterial growth curve consists of a lag phase, a log phase, a stationary phase and a decline phase. This is seen in a liquid medium.

- In **the lag phase**, the bacteria adapt to the environment. There is no appreciable increase in cell number.

- **In the log phase**, there is exponential increase in the number of bacterial cells.

-**In the stationary phase**, there is no increase or decrease in the number of bacterial cells.

- **In the decline phase**, there is a decrease in the bacterial population due to cell death.

(4)The genetics of bacteria

4.1 Introduction

-It's a part of clinical medicine, and it's used in the molecular technique in order to:

*Diagnosing infectious agents.

*Treating certain genetic disorders even malignancy.

(That we cannot understand number of diseases without genetic analysis)

- The major component of the bacterial genome is one double strand, circular DNA molecule that the tight coiling of the DNA results in a dense region of DNA called **nucleoid**, not bounded by a membrane.

***The structure of DNA:**

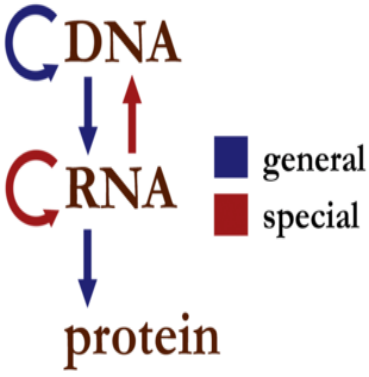
-The DNA molecule is composed of two chains of nucleotides wound around each other in the form of "double helix". Double stranded of DNA is helical.

-unlike the linear DNA of most eukaryotes, typically bacterial chromosomes are circular.{each linear DNA cannot survive in the cytoplasm of bacteria, it will be cut off due to endonucleases enzymes, and this is the reason why it must be circular to be protected}.

-Number of genes of DNA is different among species, but most types of pathogenic bacteria have about 1,000-3,000 **genes**.

Not necessary for all genes to be encoded-expressed for replication.

-1300 um long contains 2-**5 x 10^6** nucleotide bases.



***The flow of genetic information includes:**

-The replication of DNA to make more DNA.

-The transcription of the DNA into mRNA.

-The translation of mRNA by ribosomes through tRNA into various proteins, and enzymes with diverse functions.

***Definitions:**

-***Genes***: are functional units of chromosome that they **<u>are segments of DNA</u>**.

⇩

These segments might be short or long and each one of these genes can be responsible for production of one end product(polypeptide, amino acids).But, generally more than one gene contribute to informing an enzyme and bacterial structure as flagella (not only one).

*Synthesis of protein components and enzymes of cells is regulated by genes.DNA is responsible for both gene replication and function. (By knowing the combination of these genes we can know what type of end products can

Be produced by these genes).

The bacterial cell is controlled by DNA-encoded and expressed genes.

*An organism containing normal genes is known as: Wild **type.**

Genes may rarely mutate (change) resulting in heritable variation called a mutations, and the organism is called a mutant.

-Organisms can be classified into certain groups because they have certain common characteristics. Also, according to similarities in number and arrangement of genes between bacterial cells, we can classify them to Families-Genus-Species-Strains (clones), and these similarities might be in relation to genetic makeup to cell wall, presence of capsule, and others.

*The difference between 2 types of strains might be in a few number of genes in the bacterial chromosomes, and this means some of the properties (the presence of flagella, capsule, or the fermentation of lactose...etc.) will be changed and observed during the growth of bacteria especially difference in production of one or more enzymes, although they are only slight changes, because these enzymes may result with more specific characteristics to bacterial cell.

⇩

-And these differences among species may be related to:

1. (DNA (or) RNA bacteriophages >> type of bacterial viruses that have the ability to transfer genetic material from one bacterial cell to another, resulting with changes of chromosomal DNA of recipient.

2. Plasmids (Extra-chromosomal DNA)>>that can be transferred by transformation mechanism from one bacterial cell to another (it will be mentioned later in this sheet).

*SO:

- Bacterial genome includes Chromosome & DNA Plasmid, DNA / RNA Bacteriophage.

{These will result with slight changes among species which have mainly up to 95% similarity in their basic essential genes}.

#NOTE:

The changes in this case will be limited (not in the basic essential genes) that it's not easy to change G+VE bacteria to G-VE bacteria. On the other hand, you can change one single type of bacteria which can neutralize lactose to be not able to neutralize it, or from non-capsulated bacteria to capsulated.

-Genotype and Phenotype:

-Genotype=DNA (all available genes) that are considered the genetic makeup of bacterial cell. ALL properties found in these genes are not necessary to be expressed. That number of genes in most bacteria ranges between 1000-4000 but it may just 200 genes be expressed.

-phenotype=PROTEINS

Expressed manifestation of the genotype {the genes that can be expressed into cellular structures of bacteria such as: capsule, flagella, and Pilli}.

*The observed outcomes of gene expression.

*The appearance or metabolic capabilities of an organism: That it's very important in clinical medicine in order to see if there is a change in bacteria from non-pathogenic to pathogenic or change from being susceptible to

antibiotics to being resistant (resistant is acquired so it's related to phenotype).

*So, phenotype is less understandable than genotype due to rapid changes of the phenotype in bacterial cells. (Bacterial cells could convert and be resistant to some kinds of antibiotics or become toxic where before wasn't.

*Gene expression=turning the information from the gene in the DNA into molecules it encodes, usually a protein:"Not all genes are expressed: if not expressed, the genes cannot contribute to the phenotype".

-Bacterial bio-engineering:

*We can notice the major shape/appearance of genotype at the beginning of culturing.

*Any later change in the shape is the change in phenotype. Example to illustrate it, the gene type of bacterial cell is not toxic but it become toxic during maturation(inducing mutation) , and this is used for genetic manipulation(picking up some genes of specific bacteria that might give us better end products such as: lactic acid, steric acid,…) .

To sum up:

Bacterial bio-engineering: is inserting genes of interest into bacterial DNA.

*They are some segments of bacterial cells that cannot be changed: **Stable segments-------that we can use these segments in medication, such as insulin, interferon, and vaccines.**

⇩

Extra note: interferon is protein released in response to the virus entry, inhibiting its replication.

4.2 Lab diagnosis of pathogens: Polymerized chain reaction

*PCR: It's a technique used to make multiple copies of a stable segments of DNA of interest, generation a large amount of copies from a small initial sample. This small segment of DNA related to specific region of bacterial cell).

-To make it easy to understand, you can watch this animation firstly:

https://www.youtube.com/watch?v=iQsu3Kz9NYo

-Amplification of DNA segments makes possible the detection of microorganism/ cell DNA in clinical specimens (detect if these DNA is extracted from bacterial cell or human cell), then we can conclude that this organism might be related to E.coli, for example. Also, detection of pathogenic virus of bacteria as we take a sample from blood or urine, for example.

- **16S ribosomal RNA (16srRNA)**

Is highly stable region in genes of most bacterial types, that you might have one or more of these stable regions in each bacterial species and can be utilized for detection of unknown type of specimen which is associated with bacterial infection (detection of causative agent of disease), or unknown cultured bacteria that you want to know what type of bacteria it is without doing biochemical tests.

*Note:

*I n the laboratory, we can culture 95% of human pathogens. But not all human pathogens can be easily cultured so, use to detect them:

Clinical special stains, gel-electrophoresis or by using DNA technique (PCR).

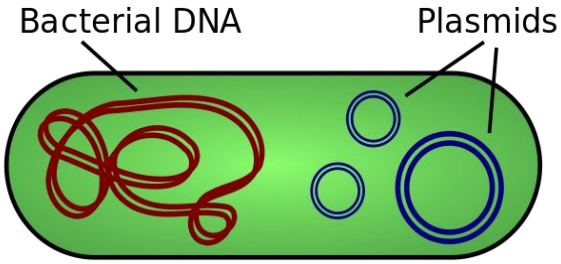
4.3 Plasmid

Prokaryotic genes are carried on:

1. Bacterial chromosome. 2. Plasmid.

#But, what is the difference between them:

-Plasmid(extra-chromosomal DNA) is : a small circular/double stranded DNA molecule within the bacterial that is physically separated from a chromosomal DNA,and can replicate independentely.



In nature, plasmids often carries additional/non-essential genes such as: resistance to antibiotics, virulent factor (the ability of bacteria to increase the

pathogenicity in host cell).While the chromosomes are big and contain all the essential information for survival.

*Due to the fact that plasmid is a double stranded circular DNA within the cytoplasm, it cannot be eliminated by endonucleases which digest linear segments.

*More than one plasmid can be found in the bacterial cells that depend on the type of bacterial cell. .. Bacterial cell contains1-10 plasmids and each contains 5-100 genes.

*__**Plasmids**__ also vary in their size.

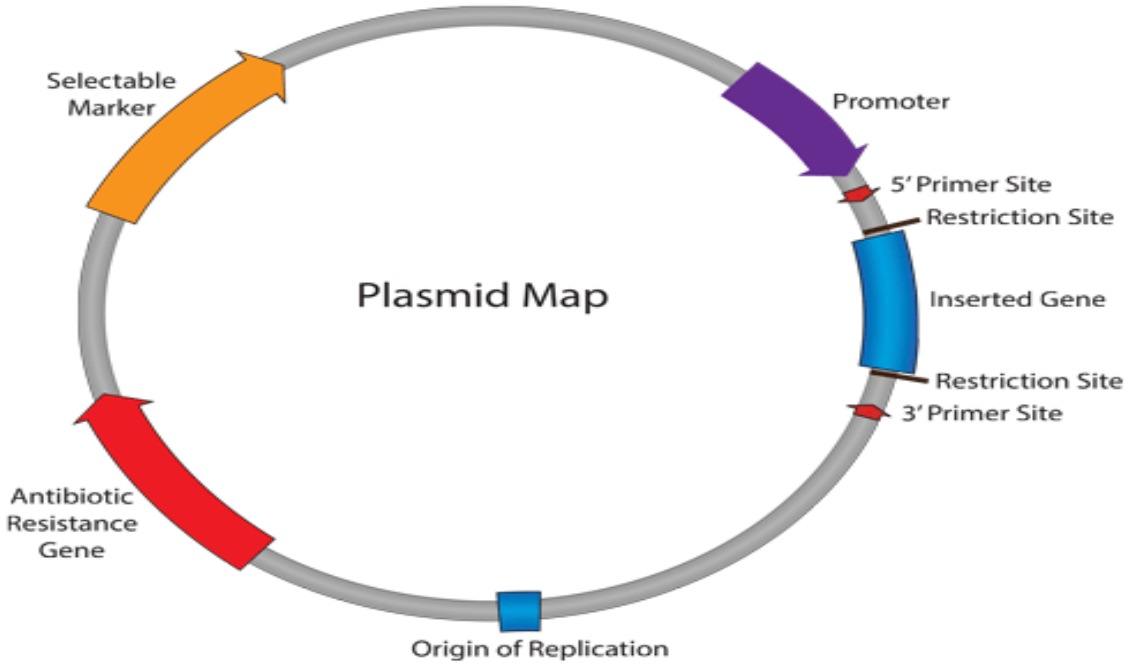
-Might be:

Conjugated

Non-conjugated plasmid

*In nature, plasmids provide one or more function benefits to the host cell such as resistance to antibiotics.

Simple plasmid



1. All natural plasmids contain an origin of replication which is a DNA sequence that allows the initiation of replication within a plasmid. (The

ability of plasmids to self-replication within the cell makes them attraction tools for the life scientists or bio-engineering).

2. (Red arrow) Antibiotics resistant genes:

Are number of genes which stand for production of enzymes associated with resistance such as beta-lactamase (ampicillin resistance enzyme) and can be inherited thus resistance **<u>can also be inherited</u>**. {The plasmid might contains one or more resistance genes}.

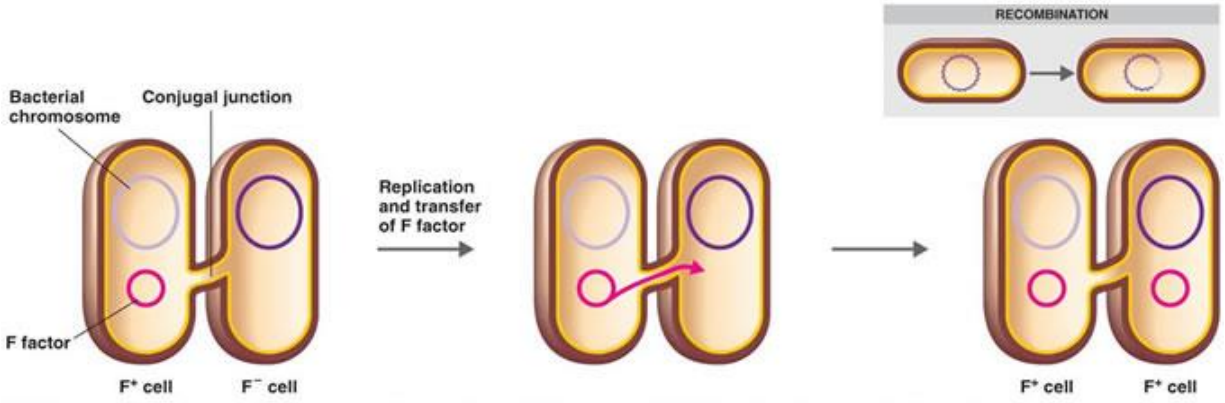
So, it opens the double helix structure and starts to release one copy of plasmid which can later produce complimentary copy (within the same cell or another cells).

*Types of plasmids:

1.Conjugative plasmids:

-To make it easy to understand, you can watch this animation firstly: http://highered.mheducation.com/sites/0072556781/student_view0/chapter13/animation_quiz_3.html

-Conjugation (we will als dicuss it later) is a mechanism of gene transfere that requires direct contact between donor and recepient cells.



(a) When an F factor (a plasmid) is transferred from a donor (F+) to a recipient (F−), the F− cell is converted to an F+ cell.

-F plasmid in this picture is fertility factor that allow genes to be transferred from one bacterium carrying the factor to another bacteria lacking the factor by conjugation.{F plasmid belongs to conjugation plasmid that control the sexulal functions of bacteria}.

- F+ bacteria are bacteria that possess F factor as a plasmid independent of the bacterial genome.
- F- Bacteria are bacteria that don't contain f factor and act as recipients.

  # when f+ and f- mixed together, eventually all the cells become f+ and in order to be transferred it requires: -enzymes -conjugation tube"pillus".

*So, f+ bacteria carry the f plasmid,enabling it to make a pilus:[a protein of bridge like connection between two cells that manage to carry plasmid between them]----f+ cells can donate a plasmid and f- cells having a lack of plasmid in their cytoblasm can receive this plasmid.

*But,conjugation needs special conditions(physiologic PH,temperature,nutrients…etc) to occur, and the presence of factors. Also, not all cells are able to accept foreign genes from other cells(from G+ve to G-ve for example that there must be certain similarity in the genotype).

*The importance of converting genes in conjugation is to convert properties from one bacterial cell to another such as:

-Toxic production.

-Antimicrobial resistance.

- Enzymes production such as Lactamase

And this results with new phenotype: that is recognized during culture/or infection.

2-Non-conjugative plasmid:

[Plamids that cannot be transmitted due to these reasons which are needed to conjugation mechanisms]:

-They don't have the mechanisms of transmition(conjugation tube:pillus for example)

-They don't have enzymes responsible for transmission.

*If there is one conjugative plasmid and other non-conjugative then the non-conjugative can cooperate with conjugative and being transmitted together.

#the plasmid is found in the G-VE bacteria more than the G+VE bacteria

And the transformation of the plasmid cannot easily happen from G- to G+VE.

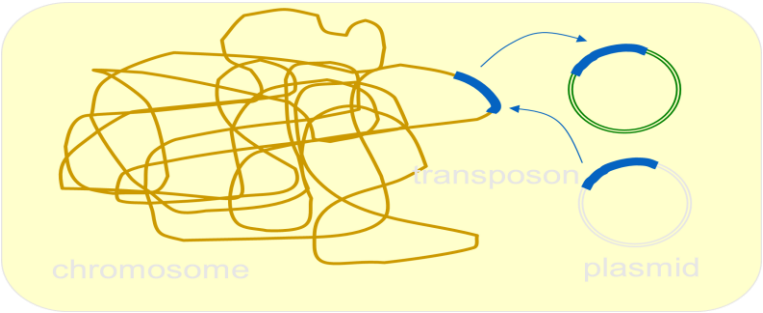
#this type is actually more abundant among bacteria than the conjugative type and often cannot change the characteristic of the bacterial cell.

⇔

So, keep in your mind that there is a narrow host range of transformation: so, there is a plasmid for E.coli and other for Salmonella…etc. And that can help in differentiation of plasmids and understanding the method of transformation between cells.

3.Transposones/Integrons(jumping genes):

-Nonessential small genetic elements that can exist in two ways in the bacterial cell: Both can be integrated into the bacterial chromosome or attached to plasmid in the cytoplasm



-To make it easy to understand, you can watch this animation firstly:

http://highered.mheducation.com/sites/0072556781/student_view0/chapter13/animation_quiz_5.html

-During the replication of the bacterial chromosome, sometimes a small segment of DNA can be seperated from the double helix DNA (but we know that the linear DNA cannot survive in the bacterial cell, so if these segmental DNA have at their ending :"insertion sequence"it can be easily to insert to another DNA bacterial chromosome or plasmid. These **segment**s are called "transposones".

⇩

-This small segment has a gene responsible for production of an enzyme called"transposase"induces the integration of the segment "transposone" to the newly bacterial chromosome or plasmid.

The importance of this process:

The scientists found that the bacterial cells once expressed to a pressure of an antibiotic they might lose these small segment (transposon) and these small segment later are usually stands for production of certain enzymes against one or more antibodies.

-Integrons :[similar to transposone but smaller than them]

They are smaller than the transposon and linear, and they are responsible for antibiotic resistance (transfer resistance markers).

#Why they are called jumping proteins?because these segments are :

*Easily can be seperated.

*Easily can be incorporated. Resulting with variation in phenotype.

4.4 Evolution of plasmid host range:

Plasmids are known to be able to move from one bacterial strain to another. However, plasmids cannot move and replicate in all strains:

-Narrow host range(NHR): (A SPECIALIST)

Many genetic elements(plasmids)seem to have specifity to a narrow range of hosts:they become increasingly better at invading and replicating in that host.(Replication without transfer but they must be attached to conjugative bacteria to be transferred)

Example: . ***E.coli , Staphylococcus*** **species** or very closely related bacteria species.

-Broad host range plasmid(BHR): (A GENERALIST)

In contrast, BHR plasmid has adapted to transfer and replicate in many different bacterial species.

-This generalist characteristics of BHR plasmid can cause problems in human society, such as the spread of multiple antibiotic resistance among pathogenic bacteria.

Thus appears tha NHR plasmids are functioning as genetic reservoir for a closely related groups of bacteria while BHR plasmids function as an active gene transporters across advirse range of species.

4.5 Bacterial mutation: (the changes in the bacterial cell)

-Mutations are heritable changes in genotype that can occurs spontaneously(simple mutation) or might be induced by chemical or physical treatment.[Organisms are selected as reference strain are called **wild type** their progeny with mutations are called mutants,and the agent inducing mutation called mutant]

-Mutation causes:

^ Spontaneous mutation(simple):

-usually happens during replication :"There is always changes in the arrangement of nucleutide bases in the genes of DNA within the bacterial cell".

-it occurs in the abscence of mutating causing agents.

- at a low frequency of **10^{-3}(<0.2)** per bacterial cell.(not easily to be observed more than 2 years during replication to detect it)

^ Induced mutation:

-DNA exposed to mutagens(chemical or physical).e.g.toxins,x-ray,UV light.

-mutagens increase the rate of mutation by possibly doubling rate.

SO, in laboratory we can make some changes on the bacterial cell but not on the major properties as the presence of capsule, and response to the gram stain as G+ or G-ve.But, we can control other things like controlling the resistance of the bacterial cell to a certain enzymes.

- it's a slow genetic process can develop in laboratory(easily recognized 10^7-10^10 changes per bacterial cell.

4.6 Mechanism of gene transfer between bacteria

- There are three mechanisms:

1- Transformation, mainly in gram+.

2- Conjugation, mainly gram- .

3- Transduction, gram+ and gram- equally.

First mechanism: ***Transformation*

-Mainly among G+ve bacteria more than G-ve.

-To make it easy to understand, you can watch this animation firstly:

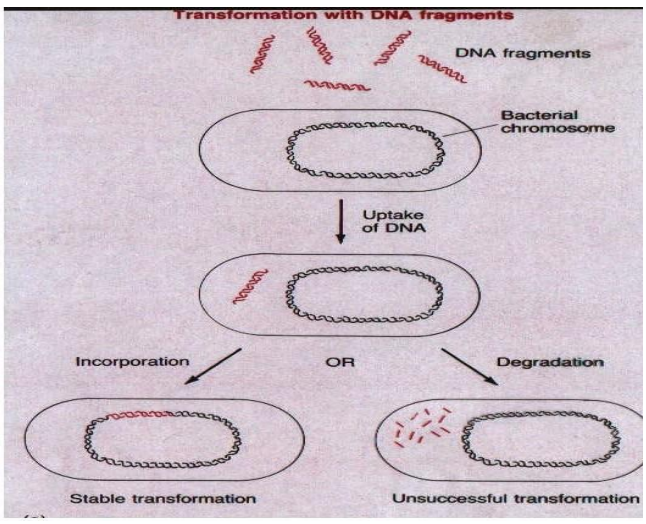
http://highered.mheducation.com/sites/0072556781/student_view0/chapter13/animation_quiz_1.html

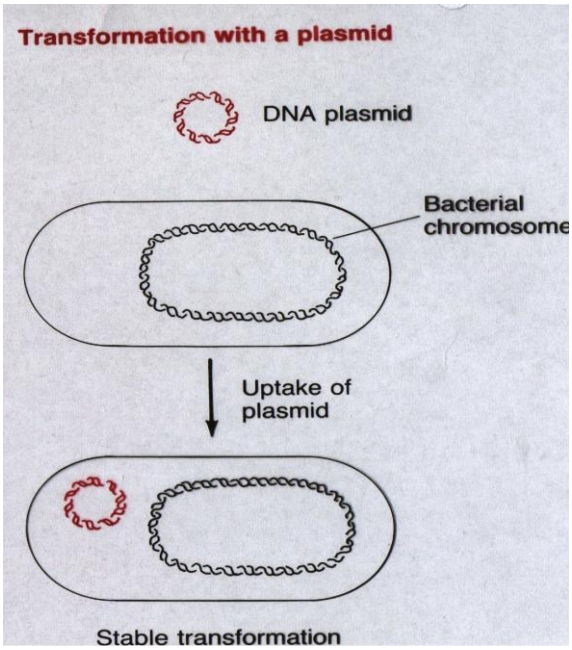
-Some of bacterial cells after undergoing the death phase, they will release all their cytoplasmic components including their chromosome, at these times other bacterial cells (which have the ability to replicate) absorb this chromosome from the dead cells, and when they inter the cytoplasm if this chromosome has the insertion sequence, then it can incorporate into the host bacterial chromosome and this incorporation occurs at specific sites (that must have certain compatibility)

So, **Transformation is: absorption of bacterial cell small specific segments of DNA which originate from other cells. (Incorporation by homologous recombination)**

#The DNA that will be transferred might be one of these two forms:

Plasmid or linear double helix fragments.





*__***These segments**** are considered as foreign segments that either (left picture)

* able to be incorporated into bacterial chromosome and be stable for long , but in this case of incorporation of these genes they might not stand forever under certain conditions that they may be separated and rejected outside the chromosome after short time.

* Not able to be incorporated that they will be eliminated by endonuclease enzymes (especially linear DNA cannot be tolerated which don't have sequence of nucleotide to be inserted).

And these small segments that are transferred carry number of genes associated with new characteristics as: Microbial resistance & Toxicity. "that can be utilized in bioengineering in synthesizing end products that may be used in treatment even malignancy treatment).

*(right picture): plasmid

_ under certain conditions, small bacterial plasmid might be incorporated but not necessarily within the bacterial chromosomes, but only in the cytoplasm …. The surrounded double helix of this plasmid allows it to survive in the cytoplasm

_ This foreign DNA might be at first linear and contains specific ''insertion sequences'' which produce pores in the cell wall and by that it will be able to reach the cytoplasm in a linear form, but due to the presence of specific enzymes that circulate the double helix …this DNA will be converted from linear to circular form (plasmid) and inherited just like the bacterial chromosomal genes during replication.

#Example of transformation:

We have a G+ bacteria in our respiratory tract called streptococcus pneumonia that is a causative agent for infection in our lung (causing pneumonia)-----And this pathogenic ability is related to capsulated form

{we might have both capsulated and non-capsulated in the respiratory tract}, and when these non-capsulated are mixed with capsulated cells during replication of its DNA, DNA segments that carry genes responsible for

capsule formation will be transferred to non-capsulated streptococcus pneumonia DNA and they will become capsulated, in other words highly pathogenic and resistant to antibiotics.

The plasmid or chromosome will transport its features to the host bacterial cell, and it will be inherited to the next generation(The majority of our intestinal bacteria change in thsis method.

#Note:

- Only certain pathogens (*S. pneumoniae*, N. *gonorrhoeae*) are capable of doing this process in vitro or vivo...under natural condition.

Second mechanism: ***Conjugation* (More complex than transformation)
-Mainly among G-ve bacterial cells than G+ve

-Bacterial conjugation as we mentioned before is:
A mechanism for transfer of bacterial genes among the same species or different species.
-To make it easy to understand, you can watch this animation firstly:
http://highered.mheducation.com/sites/0072556781/student_view0/chapter13/animation_quiz_3.html

#But, how does the happen?

1. F+ bacterium has a double helix of plasmid contains few genes responsible for formation of "Pilus: is like a bridge or tube where a copy of plasmid moves from F+ to F- "
2. Transfer one strand of the double helix plasmid
3. ✧A complementary strand to the single-stranded plasmid remaining in the donor cell will be synthesized

✧Inside the recipient cell, a complementary strand to the single-stranded DNA is synthesized

Eventually: the F- bacterial cell become F+.

#Example:

Escherichia coli: E.coli (G-ve bacteria; huge number of them 5%) that live in our intestine , and would be in certain number of F+ bacteria which are resistant to antibiotics(more than F- bacteria since F+ have resistant genes in plasmid).
When antibiotics are extensively used , they will kill most of non-resistant E.coli , then the remaining resistant F+ will replicate and increase their numbers by increasing transferring of genes responsible of being resistant against antibiotics from F+ to F-.{Including resistance to antibiotic Ampicillin}

Third mechanism: ***Transduction*

-To make it easy to understand, you can watch this animation firstly:

http://highered.mheducation.com/sites/0072556781/student_view0/chapter13/animation_quiz_2.html

-A segments of genetic material is carried from one bacterial cell to another by a bacterial virus called: **Bacteriophage or Phage.**

⇩

Is a virus that infects and replicates within the bacteria

⇩

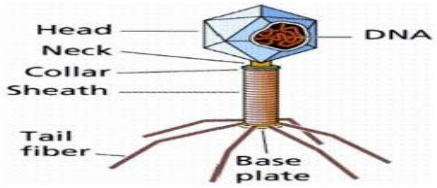
The structure of bacteriophage:

⇨ A head that contains a phage nucleic acid (DNA or RNA; NOT BOTH) packaged by (encoded protein: capsule).

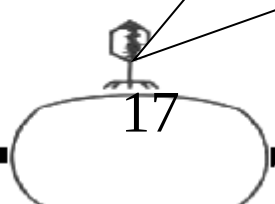
⇨ A sheath and tail fibers which provide **specific** attachment to bacteria.

⇕

* Specificity (specific characteristics of phage and specific host cell): each phage requires the presence of a particular receptor on the bacterial surface, so bacteria cells that lack the specific receptor to a specific bacteriophage is immune to infection by that:
- some bacteriophages are specific to G+ve bacteria, others to G-ve bacteria, and some of them are universal bacteriophages to G+ and G-ve bacteria.



NOTE: Accepting bacteriophage or not accepting by the host cell is called: competent and non-competent.



*****In transduction there are two methods of viral reproduction:***

1-lytic cycle (virulent phages).

2-lysogenic cycle (temperate phages). {The key difference between the lytic cycle and the lysogenic cycle is that the lysogenic cycle does not lyse the host cell}.

Lytic cycle:

Attachment and penetration: (like needle and syringe)

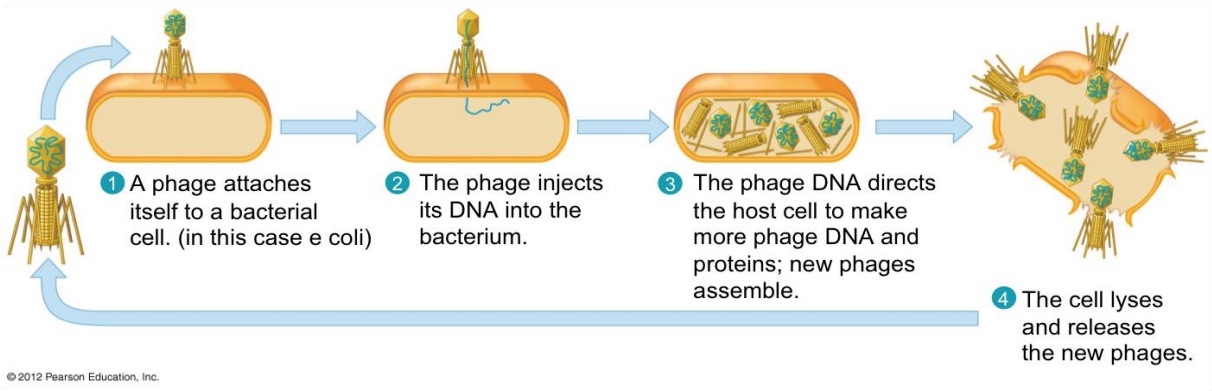
(In brief: attachment-injection-replication-lysing of bacteria and huge number of bacteriophages produced)

* To enter a host cell, bacteriophages attach to specific receptors on the surface of bacteria by its own fibres.

*Then bacteriophages will inject their genetic material into the cell, and once reaches to the cytoplasm it will become circular rather than linear to be survived from endonucleases enzymes.

* A phage enzyme is then produced that breaks down the host DNA into smaller fragments, thus the bacterial chromosome will stop the mechanism of replication, and phage DNA is replicated instead and phage coat proteins are produced (recombining the structure of bacteriophage).

* The end results will be having a huge number of bacteriophages inside the bacterial cell that later these bacteriophages will change the osmotic pressure of the host cell and produce hydrolyzing enzymes that will break down the cytoplasmic membrane and cell wall then releasing bacteriophages results with **Lytic reaction: damage of bacterial cell**



Lysogenic cycle:

-To make it easy to understand, you can watch this animation firstly:

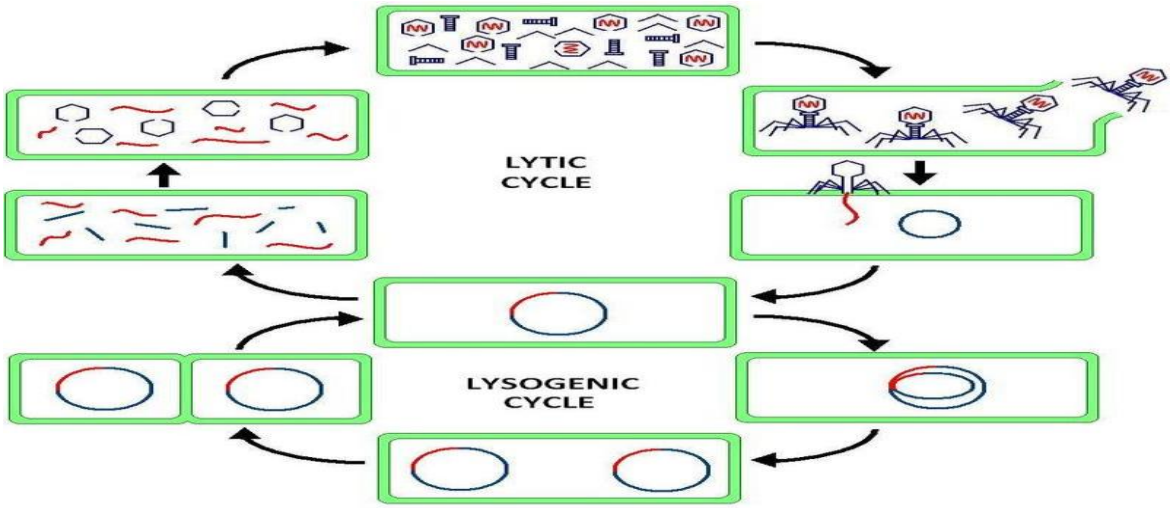
https://www.youtube.com/watch?v=_J9-xKitsd0

-It's characterized by integration of bacteriophage nucleic acid (only DNA segments) into hosts bacterium's genome.

-The genetic material of bacteriophage is called a **Prophage** that will be integrated into a circular bacterial DNA and then can be transmitted to daughter cells at each subsequent division.

-lysogenic cycle may stands for long or short time.

-Under certain conditions, the bacterial cells might get rid of these incorporated viral DNA/get excluded to the cytoplasm; which means that the lysogenic cycle becomes lytic resulting in **lytic reactions**.



-Since the prophage contains genes, it can confer new properties to the bacteria, including new end products:

* Toxigenic (pathogenic), which is the lysogenic form carrying a specific bacteriophage (which has genes responsible for the production of the toxins of the bacteria---the bacterial cell will be transferred from non-toxigenic to toxigenic bacteria).

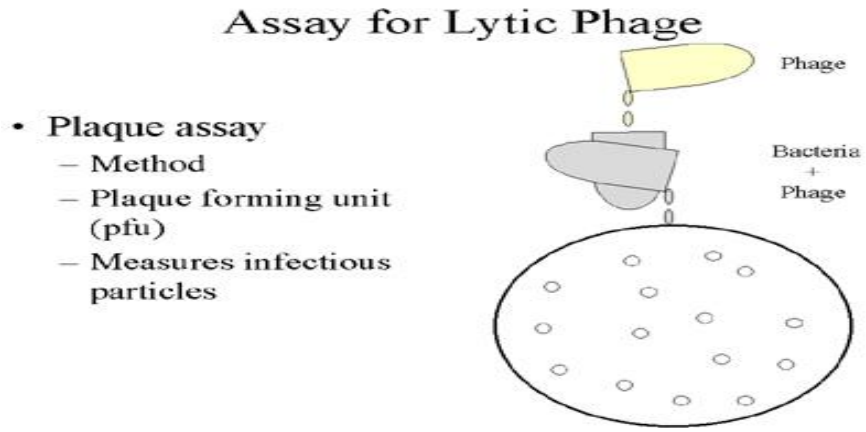
-As mentioned before there will be new characteristics and end products, due to presence of the viral genes incorporated/integrated inside bacterial genome.

#Two forms of transduction:

1. Generalized: any piece of the bacterial genome can be transferred.

2. Specialized: only specific pieces of the chromosome can be transferred:

So, it can be utilized in bioengineering that in laboratory you can examine any culture if it is infected by bacteriophages or not then using certain procedures to isolate them in order to enhance the production of specific genes to produce more certain end products, such as enzymes or toxins.



#But, how we can detect the presence of lytic phage (lytic bacteriophage)?

(Detecting E.coli –bacteriophages)

1-Isolation then culturing of E.coli until we get a heavy growth on the surface of the agar.

2-We obtain polluted water (sewage), we apply water to filtration (to obtain viruses) in order to detect the presence of bacteriophages specific for E.coli. (You can obtain then from human or animal also)

3-we put few dose of the viruses in the culture media and mix them, incubation them for hours then you will recognize the formation of plaques that you will find holes (as appear in the picture above) that indicates that the lytic specific bacteriophages are present, and the holes are due to killing E.coli by the specific bacteriophages (the holes are free of E coli cells; lysed).