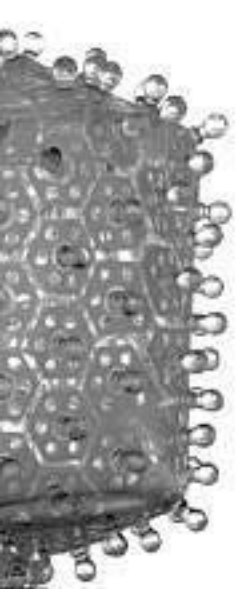


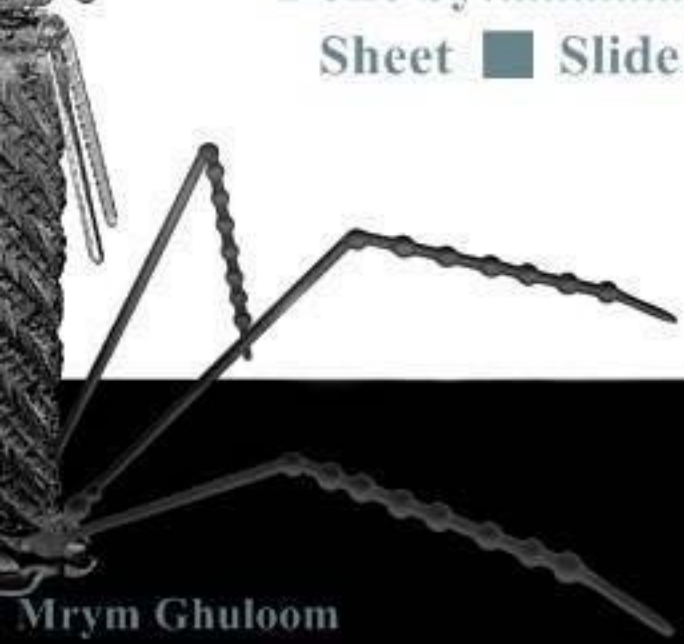
Microbiology

Lecture No: 7

Dr Name: Dr. Asem

Done by: Amneh Hammad

Sheet ■ Slide □



Mrym Ghuloom

Bacterial Genetics

♣ Before we start the lecture :

- Topics we are going to discuss In this lecture are : Genetic change in bacteria **(mutations)** , types of mutations **(natural and induced)** and mechanisms of **gene transfer** between bacteria.
- If you encountered any information that is not that much clear to you can refer to Wikipedia W or go back to the book  **(medical microbiology by greenwood pages: 69-81).**
- In this sheet I tried my best to write everything the doctor mentioned . but you still need to refer back to the slides .

Let's begin

As you remember from the last lecture we had mentioned the major basic genetic elements within the bacterial cells . mainly the bacterial genome which is composed of plasmid and the Extracellular plasmid which together usually stand for what we call bacterial genome .

These chromosomes " bacterial chromosomes " or genome and Extra chromosomal DNA which can be found in one or more part of plasmid which we have mentioned as **conjugative plasmid** which managed to be transferred from one bacterial cell to another where we have donor cell and Recipient cell. IN addition we might have **non-conjugative plasmids** which cannot be transferred without the presence of transferable resistant factors mainly or conjugative plasmid which carry the gene responsible for production of **pilus** for conjugation and later transfer of genetic material .

♣ mutations

In general we have 2 types of mutations :

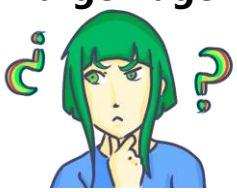
1. **Natural mutation**

2. Induced mutation

First : Natural mutation

The DNA structure for bacterial cells whether chromosome or plasmid , there might be a certain mutation and this mutation is considered as the **Natural mutation and it is a change " small change " in the sequence of nucleotide within the double helix DNA** . This mutation cannot be recognized in vitro due to few numbers of large huge numbers of bacterial cells (occur at low frequency of 10^-3 to 10^-10 per bacterial cell)

" Few numbers of large huge numbers of bacterial cells " . What is meant by this sentence ?



when you have a large number of bacterial cells let's say millions in the laboratory and you tried to recognize and to search for the cells that have the mutation , you won't be able to . That is because of the small number of bacterial cells that have the mutation . In addition , it is a natural mutation and it is made by a small change in the nucleotide sequence so the phenotype characteristics won't be that much affected . **usually this type of mutation is not controlled and not easily discovered within the bacteria .**

Always keep in mind that bacterial gene and bacterial DNA are usually exposed to some mutation " natural mutation " but often of less significant " cannot change the phenotype of the bacterial cell .

Notice that if the natural mutation is accumulated it can be associated with one or other features like:

1. Association with production of specific enzyme in relation to one or other type of anti-microbial drugs as we have mentioned before **Penicillinase .**
2. And it may also rarely be associated with certain change in the bacterial structure for example :it can synthesis and acquire capsules or slim layers to bacteria that didn't have them before ,acquire of certain adhesion factors , which allow the bacteria to attach firmly to mucosa in our respiratory tract , intestines track , etc .

Second : Induced Mutation

3. Induced mutation which is mainly applied in the laboratory where we have to use certain chemicals to change the structure of the DNA to some extent , not too much but we can change it to allow the bacteria to acquire foreign DNA.
4. *it can produce dramatic change in the phenotypic properties of the bacteria like development of resistance to antibiotics
5. This induced mutation is more important specially If associated with conjugation , transformation and transduction as we see in a few minutes and it can be recognized in **VETRO** and **VIVO** .

Questions :

1. **In vitro in the laboratory how to know ?**

Change in the characteristic phenotype specially by using a very simple test to recognize , as an example , developing of resistance to one or other type of anti-body .

2. **In vivo how to know ?**

It depends on the physician observation , if he recognize during treatment with antibodies that there is resistance to treatment and the patient is not

responding to it which means that the bacteria has developed some kind of mutations

Flying to Lisbon

2 days ago , the doctor was in Lisboan where he have discussed with other doctors the developing of resistant and one of our colleagues (a physician) who is specialist in the respiratory tract has mentioned the following :

Despite the result from the laboratory that the isolated streptococcus pneumonia was resistant to a drug called **Augmentin** "

Augmentin : (it is the trade name of Amoxicillin) **it's an antibiotic useful for the treatment of bacterial infections it contains Clavulanic Acid which is Beta lactamas inhibitor**

He told me that the result of the lab was resistant , despite this fact he gave the anti-biotic and the patient responded to the anti-biotic !!!

How we can explain this ?

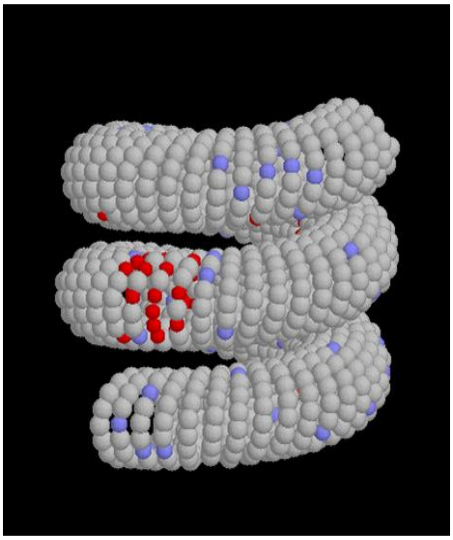
- **This is very simple to explain :**

The level of resistance in bacteria to any type of anti-biotic usually is gradually increased and not increased at once like you start of a dose of 2 mg of a certain antibiotic then you will need 4 mg to create response to treatment then 10 mg and so on ..

But in the next time it will be increased gradually in a patient who did not respond.

This means that the resistance is slowly increased , the change (mutation) is slowly not necessarily to be recognize the end product phenotype at one time it may take few weeks to be seen clinically .

So please keep in mind that the induced mutation and natural mutation might not be easily recognized but slowly it could be recognized with time and will produce problems .



This is an example . If you look here to the double stranded DNA you recognize two colors

Blue and **red**

The blue one indicates what we call **" point mutation "**

What is meant by point mutation ?

It's a mutation that is associated with the change of one base nucleotide with another in the DNA or RNA , it's affect could be silent or noticed and it could be caused by replacement , deletion , substitution Which means that it is not very dangerous .

Whereas slowly the blue is converted to red that means that the change can be recognized now whether in vitro or in vivo " clinically or in the laboratory " as a change might be associated with one or other type of resistance .

♣ mechanisms of gene transfer between bacteria.

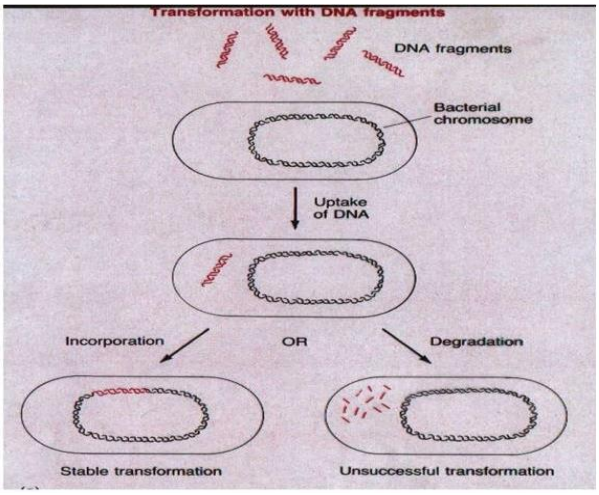
● What are the mechanisms of developing anti-microbial resistance ? and what are the mechanisms of transfer of genetic material between different types of bacteria ?

we will concentrate mainly on three mechanisms and we will not go in a lot of details but we will summarize **in a practical way** how to understand these mechanisms of transfer of genetic material.

Please keep in mind that we always repeat the developing of resistance

First : Transformation

It's the process of genetic exchange at which free linear DNA is released by dying bacteria taken up by other bacterial cells and fused to the bacterial chromosome or plasmid by Homologous recombination .



In relation to transformation if you look to this picture you will see the following :-

You have a bacterial cell , you have inside as we said the bacterial chromosome . you might have in addition as we already mentioned many times one or more **plasmids** available in the cytoplasm .

Bacteria generally not susceptible much for what we call foreign DNA , what we mean , not easily foreign DNA from other bacteria or human made can enter the cell .

But , to some extent , the cell membrane and the cell wall might allow under certain conditions the entrance of small segments of the DNA into the cytoplasm .

If this small segment managed to be integrated within the bacterial chromosomes **(this integration is a complex mechanism = not easy)** . These small segments must have too sticky endings which allow them to enter in the combination with the sequence of the DNA.

Therefore , in general , bacterial chromosome cannot accept foreign DNA which is **to some extent similar** in certain properties to the sequence of the bacterial DNA . there must be **similarity (Homology)** specially between small segment of these extracellular DNA and in any part of the bacterial chromosome . if these small segments managed to be integrated and they combined to come part of the bacterial chromosome then there is a successful transformation .

- **Notice that :**

1. **Not all types of bacteria accept transformation**
2. **Transformation is more recognized in relation to gram +ve bacteria more than gram –ve**
3. **within gram positive bacteria mainly in association with certain type of bacteria .**

As an example : **Streptococcus Pneumonia** which is found in our respiratory tract and considered as a highly pathogenic microorganism if it is capsulated.

If we have cells of streptococcus pneumonia non capsulated mixed with streptococcus pneumonia capsulated

Mixture = capsulated + non-capsulated

Note : it can be found in our **throat** or in Vitro it can also be recognized

Here and during replication if you segmented a part of the DNA of the chromosome which carries the gene responsible for the production of capsules in the capsulated streptococcus pneumonia it might manage to transfer to the non-capsulated streptococcus pneumonia DNA and transfer it to a **capsulated one.** what does that mean practically ?

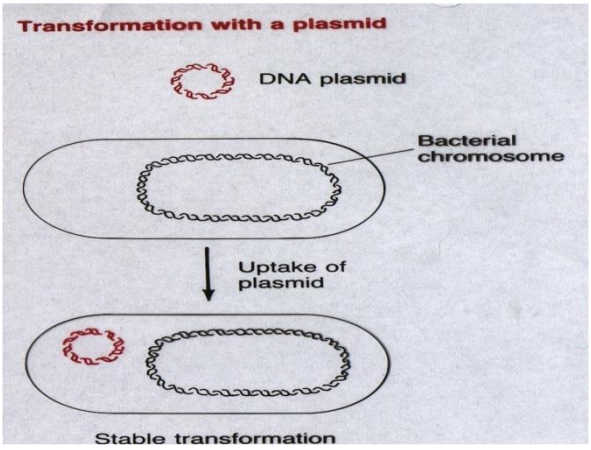
It becomes pathogenic microorganism .

● Keep in mind that in relation to streptococcus pneumonia we have about {85 capsular types}

At the end we have in transformation what we call it foreign DNA enter the cytoplasm . if managed to be included within the bacterial chromosome " usually not with the plasmid " then we have a **transformation synthesis** .

It is possible for this foreign DNA not to be accepted from the bacterial cell and so the end result will be that the nucleases **(retrenching nucleases)** enzymes will cut this foreign DNA as you see picture into small pieces and it will be eliminated from the bacterial cells .

1. Transformation of plasmids



transformation can also be used by the use of plasmids. Instead of attachment to the bacterial chromosome it might be carried on a special plasmid and here you first introduce a first segment of DNA into this plasmid and then try to introduce this plasmid into the

cytoplasm .

Questions :

1. In vitro in the laboratory how to follow ?

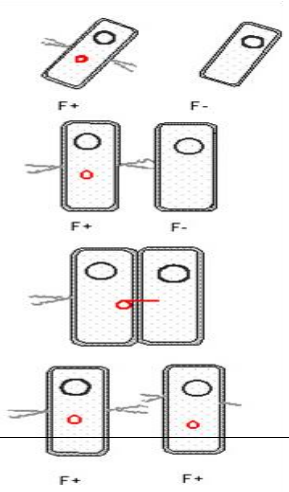
This is a process that requires certain condition in VITRO like using : calcium ions and other ways of manipulation .

1. In vivo how to follow ?

In vivo in our body it cannot be followed.

- **Very important note:**

1. This type of transformation with plasmid is found in **both** , gram +ve and gram -ve but **mainly** in gram +ve .
2. Remember that the transformation associated with the bacterial chromosome is **only** related to gram +ve .



Second : Conjgation

Conjugation as you see In this picture . we have cells with the F factor " F+ cells " which are cells that carry a plasmid and this plasmid carries specific genes responsible for developing

of conjugation through **production of Pilus** . as later , this gene is transferred as small segment of DNA to susceptible F- cell (don't have that plasmid) . so we have donor F+ and recipient F- .

● **As you see in the picture above :**

1. The first step usually occurs by transcription and translation within the plasmid to produce necessary enzyme and the necessary Pilus which brings two cells together .
2. The second step , one copy of this plasmid will be transferred . In result , The F- cells that were without plasmids now they will acquire plasmids with genetic information similar to the F+ cell .

This is highly important specially for dissemination of antimicrobial resistance and for transfer of toxins .

Example : gram –ve enteric bacteria found in the intestine which are types of bacteria which resides in our intestines and they are important for us as a commensal bacteria .

examples of it : E-choli and many types that you will take later but at the moment keep in mind the gram –ve enteric bacteria.

This cellular process of conjugation in fact is the **most important feature recognized in the relation to the development of anti-microbial resistance or evolution of anti-microbial resistant in our days** .

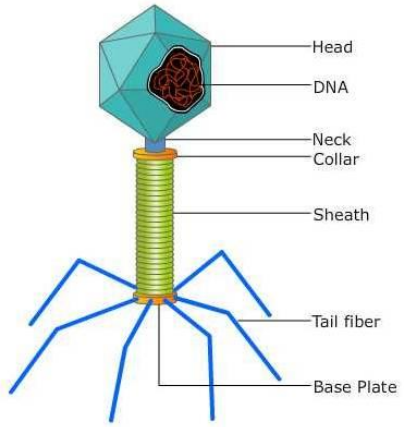
Third : Transduction

Transduction it also means transfer of genetic material , not directly , and not by presence of conjugative plasmid **but through a specific virus known as Bacteriophage** : which is a virus that infects and replicates within the bacteria .

• Before giving you exact features of this Bacteriophage , please keep in mind:

For each type of bacteria there is a specific Bacteriophage . It is rare to recognize one type of Bacteriophage which can infect many types of bacteria . **(many types of bacteria = genes of bacteria) .** there is certain specificity in Bacteriophages .

If you look here to the structure of this Bacteriophage what we see is a simple structure composed of :



1. Head which contains DNA or RNA but not both " just like any other viruses "
2. Special Sheath used for absorption to obtain fluids or for the injection of fluids . this sheath , inside is like a tube and there is a base plate as you see in the picture .
3. Tail fibers

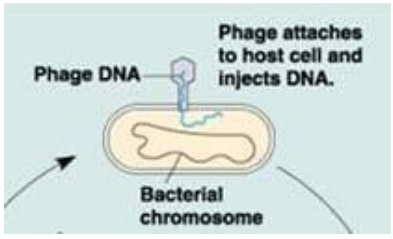
The head is surrounded by a protein coat and the fibers also and the base plate are composed of protein.

These tail fibers with the base plate attach on the surface cell wall of the bacteria , attaches firmly → remember that it attaches with certain specificity " some bacterial cells can accept the bacteriophage while other do not **" it is called competent and non-competent .**

If it manage to be fixed on the call wall (has a place on the cell wall) and once it attached then it can manage to inject their own DNA . once the DNA is injected inside the bacterial cell , as you see in the picture we have **2 features (stages)**

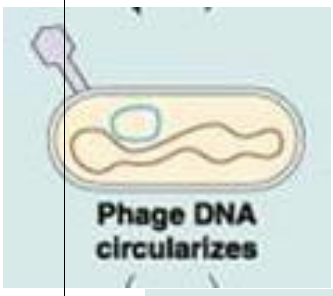
Lytic cycle (lytic end product) and lysogenic cycle

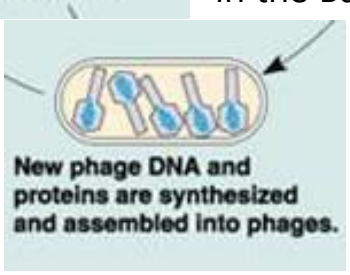
♣ Lytic cycle



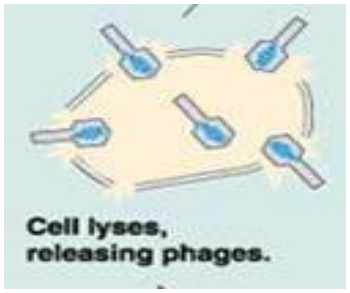
1. As you see in the picture the Bacteriophage will attach to the cell wall of the bacteria , injects it own DNA .

2. once it reaches the cell chromosome it begin to produce necessary enzyme to break down the bacterial chromosome and any other DNA within the bacterial cell and the enzyme which is produced in order to break down the bacterial DNA is a polypeptide produced by the mechanism of transcription and translation which is coded in the Bacteriophage DNA.





3. within a short period these bacteriophages will become huge in number inside the bacterial cell .

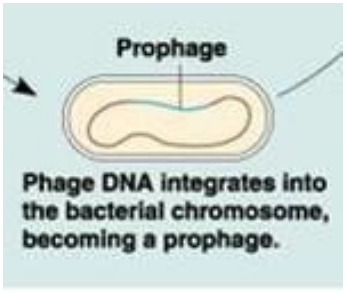


4. The new formed bacteriophage will produce hydrolyzing enzymes that will break down the cell wall and membrane of the bacteria resulting in a release of a huge number of new bacteriophage .

This means the end result of the Lytic cycle is : Damage of cell wall and cell membrane and release of new Bacteriophages.

The end result is causing infection and the kill of the bacterial cell associated with the production of new bacteriophages .

♣ Lysogenic cycle



On the other hand , there is another type of phages called **Temperate phages** (temperate : مؤقت) which in contrast to the lytic phase , the phage DNA as you see will be integrated within the bacterial chromosome . It becomes a part of the bacterial chromosome . In lysogenic form (مرتبط) the viral DNA is integrated within the bacterial chromosome and begin to be replicated exactly with the bacterial chromosome but not for indefinite time .

This lysogenic stage or lysogenic cycle in fact might survive for many generations with the bacteria , for many weeks , months without damaging the bacteria .

BUT

might by the presence of specific triggers (environmental conditional triggers and physiological changes) might result into developing into lytic stage.

Lysogenic stage retain to become lytic stage

The lysogenicity in certain type of bacteria is associated with the presence of certain specific enzymes or toxins and specially toxins that affect certain types of microorganism .

Example :

We have in our throat " our respiratory tract " mainly in association with mucosa , there is certain number of what we call **Corynebacterium Diphtheriae** which is a pathogenic bacteria that causes **Diphtheria** and it is very dangerous disease specially for children who lack a well developed acquired immune system .

In Jordan as you know we have 100% immunization against many diseases .

If a person for certain reasons did not receive the vaccine or during his life his immunity decreases he might encounter corynebacterium diphtheriae which will produce toxigenic materials .

note that within our respiratory tract we have non-toxigenic corynebacterium diphtheria and it can be associated with a few number of toxigenic corynebacterium diphtheria and in any certain condition if a bacterophage reaches the toxigenic type it will then spread in large numbers and convert the non-toxigenic corynebacterium diphtheria into toxigenic and cause diphtheria .

This is the importance of lysogenicity

In addition , using lysogenicity or temperate phage is widely used in bioengineering in order to produce specific type of genes in certain type of bacteria to produce a useful end product or to change the characteristic of bacteria .

So the lysogenic is an important feature of bioengineering as well as in nature and both **(lytic and lysogenic)** can be recognized within our human body

It is difficult to say now which is more important . Each one of them has certain important features in our body but the lysogenic **is with more significant importance** . The lysogenicity may be also associated with many systems in our body.

as you have seen we have three mechanisms : Transformation , transduction and conjugation might associated with the transfer of necessary genes from resistant bacteria and this will contribute in development of resistance .

♣ The transduction is of 2 types :

1. **Generalized :** the Bacteriophage can introduce its DNA to any part of the bacterial chromosome .
2. **Specialized :→ (only specific pieces of the chromosome can be transferred) .** sometimes is required for bioengineering to introduce specifically the Bacteriophage to a specific site on the bacterial chromosome in order to enhance the production of specific genes to

produce more of certain type of enzyme which can be specially used in bacterial genetics and studying of certain bacteria to recognize certain end product . we might have many useful products like certain types of polymerases and endonucleases by the manipulation of the bacterial genes by the method of specialized transduction not generalized .

What the doctor mentioned above are hints . there are more details about these 2 mechanisms but we are not required to know these details

A Question :

How we can in our laboratory (in VITRO) to recognize if there is a bacteriophage ? or in other words , how we can study a certain type of bacteria by using of lysogenic or non-lysogenic Bacterophage ?

The mechanism is known as **plaque assay**

Note : the information mentioned bellow are taken from a website . what the doctor explained was not that much clear specially that some words were not understood . I will refer back to the doctor for more explanation . sorry for that and enjoy reading what follows .

The purpose of the plaque assay is to grow isolated plaques of phage particles within a lawn of bacteria. Students can visualize the clearing of bacterial growth on the agar media, demonstrating the effect of phage on bacteria. This method also requires that students perform serial dilutions, and allows them the opportunity to apply a concept that most find difficult to grasp.

Aliquots of diluted bacteriophage are mixed with host bacterial cells in several milliliters of soft agar, which are then spread onto agar plates containing media. The use of soft agar allows the phage to easily diffuse through the medium giving more consistent plaque formation. It also eliminates the problem of

uneven absorption of the bacterial-phage solution into the hard agar that often caused uneven plaque formation on the plate .

The bacteriophage adsorb onto the host bacterial cells, infect and lyses the cells, and then begin the process anew with other bacterial cells in the vicinity. After 6 – 24 (the doctor said from 24 to 48 hours) , zones of clearing, plaques, are observable within the lawn of bacterial growth on the plate. Plaque characteristics are related to the type of bacteriophage as well as other physical and chemical characteristics of the system in which the bacteriophage are grown.

♣ The message of the doctor to us from this lecture is the following :

Only few hints were mentioned and you need to read more and more about the topic of bacterial genetics . this lecture purpose is to give you some idea before we enter with our next topic " Anti-microbial agents " .