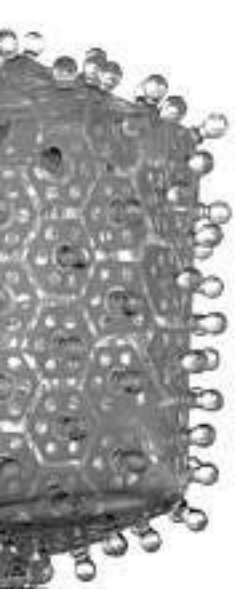

Microbiology

Lecture No: 5

Dr Name: Asem Shehabi

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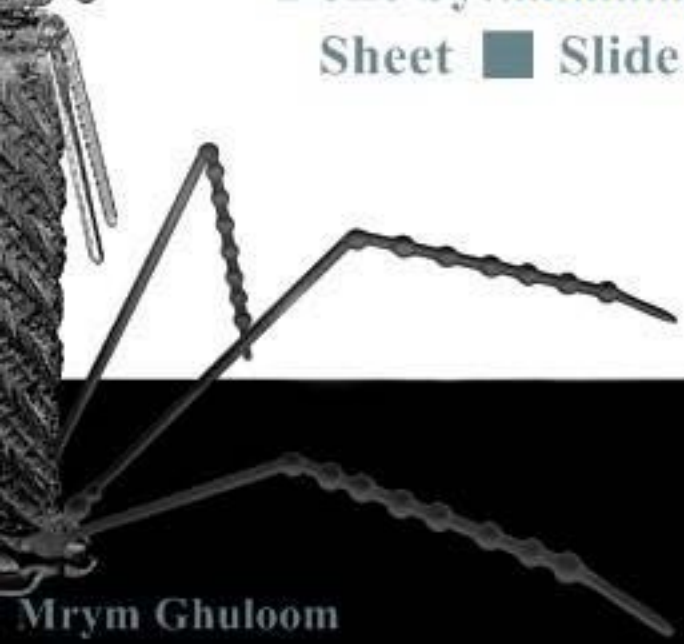
Sheet ■ Slide □



Mrym Ghuloom

Bacterial Genetics

This lecture starts with a new subject called bacterial genetics, every word is important to know and understand.

- Bacterial genetics is important for many **reasons**:

(1) Anti-microbial resistance.

This reason is a practical one, which is important for clinical diseases and infection in relation to developing new types of bacteria which are resistant to antimicrobial drug.

Nowadays it's very difficult to treat infections with one type or more of antibiotics. On the other hand, fifty years ago, treating infections was much easier with penicillin or any other drug. Now, a large number of bacteria that cause infections are becoming resistant for antibiotics, and nobody knows whether in the next few years it will be very difficult to treat certain kind of infections that may lead to death.

(2) We have discovered that bacteria always are developing mutations within the bacterial chromosomes, and this mutation is associated with transfer of genes or a change in sequence of genes within the bacterial chromosomes. This means that some non-pathogenic microorganisms are converted to become pathogenic and toxic or at least it becomes important in clinical medicine due to the production of one or more enzyme or end product. Therefore bacterial genetics will explain how the features of bacterial genes can be formed, transferred, and changed from normal to abnormal and so on.

-In the beginning of this course, we have mentioned that all prokaryotes (including bacteria) carry one chromosome (composed of double stranded DNA) in the circular form.

Written by Bana Al-Mikhi

In biochemistry, you studied that DNA's basic unit is nucleotide (Adenine, Guanine, Thiamine, and Cytosine), this structure of DNA is a component of bacterial cells, and any change in the sequence of DNA usually results in mutations.

*Mutation: any change in sequence of nucleotides within the double stranded DNA.

-The genome of any bacteria must contain genetic information encoded on the double stranded DNA in order to replicate and grow. In the process of biological activation of bacterial cells according to type of genes, the bacteria starts to produce important components of growth, as we talked in the last lecture any production of proteins, for example: the protein needed for adaptation to temperature needs information that is encoded on the DNA.

*So to conclude, genetic information is needed to produce compounds that support growth. **BUT** it is not necessary that **all** genes are translated in growth, maybe we only need about 1 to 100 genes (to produce the necessary components, proteins, and other structures in the cell)*

*Bacterial genome:

- Composed of a long double stranded DNA.
- Length approximately is about 1300 μm.
- DNA is circular and coiled, concentrated in bacterial chromosomes.
- Contains 10^7 - 10^8 nucleotides, but generally most types of pathogenic bacteria carries about 1,000-3,000 genes.
- Not necessary all the genes to be expressed or encoded for replication for example.

In brief (the doctor said that in biochemistry we must have taken the whole DNA structure), genes must be translated and then transcribed by a number of enzymes, such as DNA polymerase or RNA polymerase, all of these steps will result in the production of proteins and central dogma.

***please refer to the slides for further information about this.*

- The basic unit of chromosomes is genes, and genes are segments of DNA, and this segment of DNA is like a code transcribed and translated into a final product which is polypeptides (of course this needs replication of DNA).

Linear DNA cannot survive in the cytoplasm of bacteria, it will be cut off due to endonucleases, and this is the reason why it must be circular to be protected.

It's important to know the sequence of DNA in order to classify the bacteria into families, genus, species, and strains (clones). In the species, there might be two or more strains that differ only in the production of one or two enzymes, although there is only slight changes they still are called different strains, because these enzymes might result in more specific characteristics for example:

1- a change in **color** of bacteria.

2- or the enzyme might be associated with the **production** of flagella, pili, or capsules.

3- or change in **heredity** of bacterial cells.

- Bacterial chromosome is to some point stable. Despite this stability, there is a change in the sequence of DNA during replication, this change is sometimes silent and cannot be easily discovered in expressed characteristics, the change must reach a level to be recognized.

-Types of mutation:

(1) **Natural**: usually not controlled and not easily discovered within the bacteria culture.

(2) **induced**: usually controlled, let's assume you have a bacterial culture and you want to increase number of genes that are responsible for the production of specific enzymes, you can do that by using mutagens, chemical agents, UV light, or whatever.

- Bacterial genome is composed mostly of stable genes within chromosomes, and this can be referred to genotype or wild-type.

What is genotype?

Each type of bacteria has a well-known set of genes, these genes are of two types, one of them can be expressed to produce necessary polypeptides. The genotype is all available genes in bacterial genome. (but not all genes can be expressed)

What is phenotype ?

phenotype is the genes that can be expressed to a certain cell structure such as; capsules, flagella, and pili . It is important in clinical medicine in order to see if there is a change in bacteria from non-pathogenic to pathogenic or to change from being susceptible to antibiotics to being resistant (resistant is acquired, so it's related to the phenotype).

For example: a change in response to ampicillin drug is due to phenotype.

- Note: the phenotype can be observed in lab, by production of color or fermentation of sugar.

- Genes in bacteria can be manipulated especially in research laboratories to be used for production of useful materials, therefore, **bioengineering** now is one of the most common methods of producing new products including certain medication, such as insulin, interferon and vaccines, by manipulation of bacterial cells, in fact by getting specific genes from a certain type of bacteria and introducing it to an another type and do combinations of gene arrangement.

- In the past, if we had a patient suffering from a disease, we will have to get a sample from the patient like urine or blood, then we have to culture it and wait 24 hours to recognize the culture and do biochemical tests that may require 2-3 days, at the end we'll try to decide if this organism is a staphylococcus, or group A streptococcus, or another type.

But nowadays, there is available knowledge about the sequence of DNA, in many types of bacteria we can use very simple methods called **Polymerized Chain Reaction (PCR).**

In short, within the double stranded DNA, we have variable stable segment of DNA which is found in all species of bacteria, this segment can be used to identify directly, without the need of doing culture, the organism, usually we compare primers (small segment of DNA related to specific regions of bacterial cells) and used to produce double stranded DNA combination of one copy from infected blood or urine of the patient for example, with the available segment, and from this we might conclude that the organism is related to E.coli for example.

Of course we use a lot of techniques like gel-electrophoresis, special stains, equipments that also help us in measuring the size of sequence according to number of nucleotides usually measured in kilobase (Kb). All of these mentioned techniques are usually used in what we call **PCR method** or **PCR in association with gel-electrophoresis**.

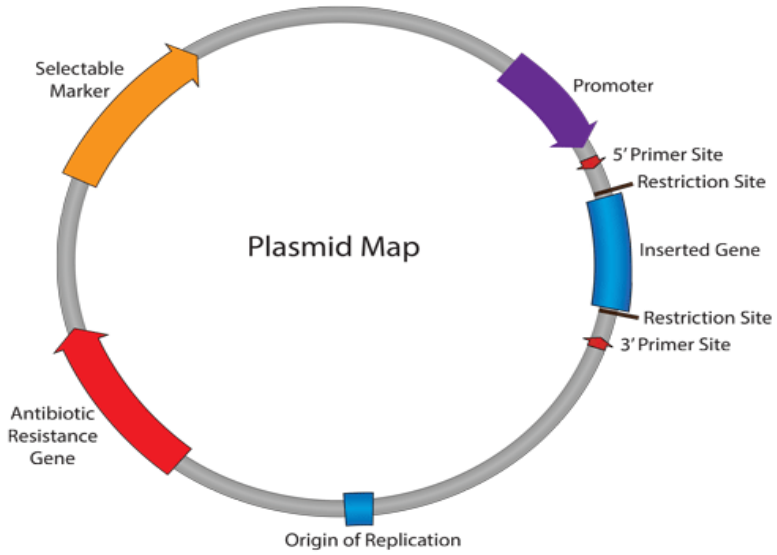
- Stable regions in genes of bacteria is called *16S ribosomal RNA genes*, each species of bacteria have one or more of these stable regions and can be utilized for detection of unknown type of specimen which is associated with bacterial infection, or unknown cultured bacteria that you want to know what type of bacteria it is without doing biochemical tests.

*Plasmids :

within bacterial cells we have something called plasmid

- it is defined as **extra chromosomal** double stranded DNA.
- it is **independent** in replication by chromosomes.
- it is **NOT DNA necessary** to duplicate or replicate an exact copy of bacterial chromosome, it might replicate it less or more depending on it's efficacy.
- it is found in the cytoplasm in **circular** form.

- if it is a simple plasmid, it'll be composed of approximately 40-50 genes divided in parts, the most important part is the origin of replication, if this origin is not available then the plasmid can NOT be replicated.
- another part on the plasmid is called **promoter site**; which allows to attach a small segment or release a segment from the plasmid.
- **restriction site** for integration of small segment of DNA, because this plasmid might acquire a small segment of DNA from the cytoplasm of bacteria which might be released from the acquired chromosome and associated (it's usually 2 or 3 genes).
- Plasmids play a role as culture for what we call: **resistant plasmid**. In the resistant plasmid there is a small segment responsible for antibiotic resistance, these express in the form of enzymes. Usually it is a small circular DNA associated with the plasmid and for example responsible for production of enzyme called β-lactamase (or also called penicillinase enzyme) that breaks down the antibiotic penicillin or any drug related to penicillin, like amoxicillin.
- It might be only one resistant factor, or two, or three. For example, we have a small segment responsible for pencillinase production or another for tetracycline resistance and this is important and can be incorporated in the plasmid.



Therefore; resistant plasmid is important to change the phenotype of bacteria. If we had a cell of bacteria, and this cell carries one or more plasmid.

- NOTE : *Within any bacterial cell, any plasmid can be found in one single copy or up to 1000 copies, as the number of copies increases it will be more difficult to treat this bacteria with an antibiotic, and the plasmid can be recovered easily and easier to tell its characteristics, because during the preparation of DNA it will be easier if the DNA was larger.*

The number of copies is important in relation to bacterial cells, but it will NOT change a lot in the general bacterial characteristics.

- Another feature of plasmids, some of them can remain only in the cytoplasm and cannot be integrated in the bacterial chromosome, these are independent group of plasmid, and we have a plasmid which can manage to become part of the bacterial chromosome by recombinations called **episomes** (instead of naming it plasmid, we use the term episome).

Episomes contain the majority of genes, including the genes responsible for the production of toxins or any type of end-product.

Plasmids usually do not carry essential genes for the growth of bacteria, instead they carry genes that change the phenotype and not the genotype. They carry genes for production of less important specific enzymes.

Types of plasmids (according to type of genes) :

1- Conjugative plasmid: can integrate by the presence of pilus (sexual pili/ 20 appendages in each cell), manage to carry one copy of the plasmid from one cell to another.

The cells must be F+ (F stands for fertility - donation of genes), conjugative plasmid can carry the copy of plasmid from F+ to the F- cell.
If F+ is connected to F- cell, the end result will be 2 F+ cells and one copy of the plasmid.

Each type of bacteria cannot accept any conjugative plasmid; there must be a certain similarity in the genome.

This process (conjugation) is actually not this simple and occurs at low frequency, as it needs special conditions and the presence of certain factors

For example, inside our intestines (large intestines), the presence of a large number of cells within a closed system and the physiological conditions of pH, temperature, nutrients, etc... Allow conjugation to occur.

For these reasons, conjugation is recognized in our intestine more than in our oral cavity, or skin, and often results in developing of antimicrobial resistant bacteria, and it's a problem, especially during administration of drugs.

In nature: polluted water/ sewage have huge amounts of bacteria and close contact between different strains and therefore conjugation can happen.

However, not each type of bacteria can accept any type of conjugative plasmid or foreign gene, there must be there must be certain similarity in genotypes.

Ex: conjugation happens relatively easy between different strains of E.coli but it is less likely to happen between another type of bacteria and an E.coli (though it can be done in the lab).

2- Non-conjugative plasmids:

Some plasmids cannot be transferred via pili, they are called no conjugative plasmids, they must first be attached to a conjugative plasmid to be transferred, if they are present alone in the cell without the presence of another plasmid that is conjugative they will merely be silent plasmid available in the cytoplasm. This type is actually more abundant in bacteria than the conjugative type and often cannot change the characteristic of the bacterial cell.

The presence of non-conjugative plasmid does NOT make the cell a male cell (f+), it is only called f+ under the presence of the conjugative type

Transposon:

A small segment of DNA which can be integrated due to the presence of integrating enzyme within the plasmid. It is composed of a few important genes. (We're sorry we could not hear the doctor's voice in the recording from 39:42-40:04, we think that he was reading something strange which was written in the slides and the slides were not yet given to us).

Integrons: (similar to the transposon)

Small segment double stranded DNA normally linear can be found during replication and cannot survive for long without integration back to the chromosome or plasmid, otherwise they will be broken down by nuclease.

All transposons and integrons are not essential for growth of bacteria, but they produce specific phenotypes, mostly related to resistance of antimicrobial pesticides.

These small segments of DNA are called Jumping genes, because they are often separated from double stranded DNA in small segments and later integrate into plasmid or into chromosome. Their importance is acquiring a specific gene which codes for a specific enzyme ex ***penicillinase***

We have a wide range of plasmids, many types of plasmid can be transferred from one species to another, but there are specific types of plasmid which are related to specific types of bacteria, some plasmids are found in gram negative bacteria , some in gram positive and some in both. Most plasmids of clinical importance are usually related to one species of bacteria. In the lab, we can control these mutations and utilize plasmids to carry genes from humans like the process of production of insulin and so on (bio engineering)

Not every time there is replication there is a mutation

A donor cell has what is called F plasmid or resistance transfer plasmid programmed to donate one copy of the double stranded DNA and the recipient, the importance is in developing new phenotypes of bacteria, raising new types of bacteria resistant to new antibiotics, also, a bacteria might produce a specific toxic end product.

If there is an outbreak of a new disease in an area we need to know the strain of bacteria and the mutation that gave rise to it and its pathogenic properties. Also a mutation can change the antigenic structure and therefore change the immunologic response so the genetic variation is important in the production of vaccines.

Note: Please refer to the slides and check the figures; we didn't put any figures in this sheet as the slides were not given to us.

Special thanks to Yanal Al-Omari who helped me in this sheet.