Microbial Genetics

Third Step **Assit. Prof. Alaa Naseer**

Lab.1

Science deals with studying the inheritance of microorganisms and branches to bacteria genetics and viruses. When studying the inheritance of the heterogeneity of the organism it is necessary to identify all factors that help to understand the mechanisms of this object . Scientific facts have shown that genetic factors in bacteria and higher organism are concentrated in specialized area in linear form , these factors can transfer from cell to another by several ways. The changes that occur in the qualities of the bacteria as a result of recombination or mutation often related with components and functions of many parts of the cell. Scince these information responsible for these traits coded in microorganism genotype , known genome the sequence of DNA strand consisting of nucleotides. At the beginning of the last century knew little about the DNA while the proteins were the only complex molecules in the cell , it was thought to be responsible for the transfer of genetic information , but the information that were known raised doubts about this belief , it was observed that all the cells of a single organism containing equal amounts of DNA while the various groups of cells in a single organism containing different types and amounts of proteins , which led to draw attention to the DNA material being nominated to carry the genetic information .

Scientific experiments have shown that DNA is the genetic material :

- Frederick Griffith experiment 1928 which discovered the phenomenon of transformation

- Avery, MaCcarty and Macloed experiment 1944 which showed that the DNA is responsible for transformation in bacteria .

- Hershy and Chase experiment 1952 which proved that DNA is responsible for genetic characteristics transfer in bacteriophages
- Chargaff discovery or Chargaff law 1947 which explain the nitrogenous bases rates A%=T% , C%=G% and the percentage of these rates vary from one to another organism .

- For the first time in London laboratories Rosalind Franklin 1952 filmed DNA by X-rays which appeared the helical structure of DNA .

- Watson and Crick 1953 who described the construction of the first DNA sample depending on Chargaff law , this sample showed the nitrogenous bases binding C≡G , A=T , also shows that DNA chains or strands corresponds one to another in opposite direction one of them with 3′ end while other with 5′ end .

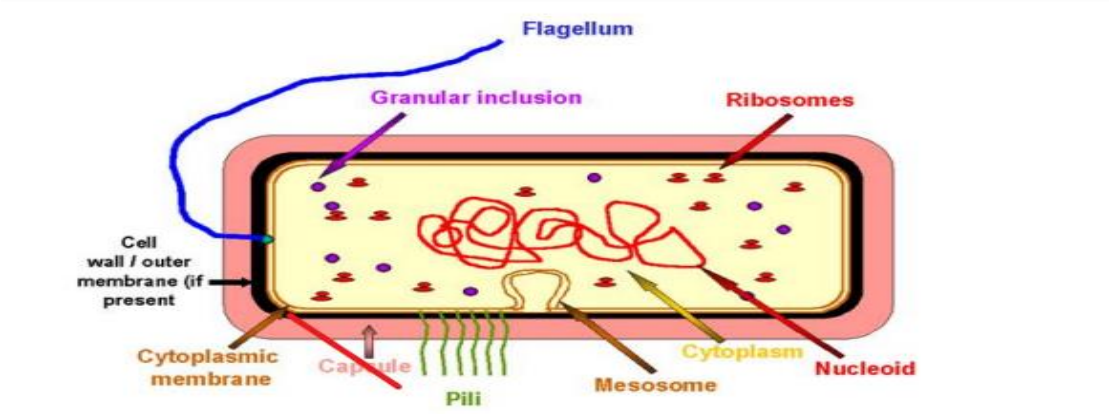
There are some types of bacteria , such as Bacillus subtilis , E coli ideal organisms for the study of DNA and genetic processes because of the simplicity of installation and short generation time , which saves a lot of time and effort , especially if the installation of DNA is similar between organisms if they prokaryotic or eukaryotic . Bacteria is a single –cell microorganism surrounded by cytoplasmic membrane and cell wall . Cytoplasm contains the ribosomes which responsible for protein synthesis in addition to a number of soluble enzymes , as well as cytoplasm contains the genetic material DNA spreading in the cytoplasm because it doesn't surrounded by nuclear membrane so it called prokaryote. The whole genetic material in bacteria or genome is a single chromosome which is circular double helix but swirled on so cannot observed the beginning from the end . In addition to the chromosome bacterial cell may contain genetic factors outside the chromosome called plasmids which is a bout small pieces of double circular DNA and have the ability to self – replication ,

these plasmids carry genes not essential for life or growth , but give additional characteristics to the cell which has these plasmids may be important to the bacteria in certain cases such as
a- Antibiotic resistance by Resistance or R-plasmid

b- Fertility or F- plasmid

c- Colicin production by colicinogenic or col –plasmid .

d- Antibiotic synthesis plasmid. e- Heavy metals resistance plasmid.



DNA extraction :

Most DNA extraction protocols consist of two parts

1. A technique to lyse the cells gently and solubilize the DNA

2. Enzymatic or chemical methods to remove contaminating proteins, RNA, or macromolecules While In plants, the nucleus is protected within a nuclear membrane which is surrounded by a cell membrane and a cell wall. Four steps are used to remove and purify the DNA from the rest of the cell: Lysis,Precipitation, Wash, Resuspension.There are nucleic acids in living cells are interconnected with proteins as they appear in the cell is a Nucleoprotein complex, so begin extraction

first process of cracking walls or cell membranes are careful to allow the exit of DNA and other cellular components without

exposing them to significant damage, then place the process of separating and extracting DNA from these complexes (proteins) through the process of removing proteins (Deproteinization process) involving three transactions :

1) Enzymatic Treatments: These transactions involve the use of proteiolytic enzymes such as proteinase K & Pronase that lead to cracking molecule of protein to short peptide chains to facilitate removed in subsequent transactions.

2) Chemical Treatments: which divided in three treatments:

 Chelating agents such as EDTA (Ethylene Diamine Tetra Acetic

Acid), It has the ability to pull and remove ions bilateral parity

Ca++ & Mg++ That contribute to maintaining the stability of protein complex and the stability of nuclear and cellular membranes, Inaddition to being catalysts for the effectiveness of enzymes Nuclease.

 Detergents agents: such as SDS (Sodium dodecyl sulfate) of ionic detergents and Sarkosyl you prefer to use on the SDS because the latter works on cracking small pieces of DNA either Sarkosyl be few cracker.

On the other detergents Tritonx-100 which is non-ionic detergents. Detergents considered highly effective crash factors acting on breaking peptide bonds and thus disengagement amino acids.

 Treatment organic solvents: such substance Isoamyl alcohol,

Chloroform, Phenol: acting with Two- Phase system, when treatment Lyset cell or cell lysis, these solvents or a mixture of them in addition to helping denaturation and remove the fat,

they lead to the formation of several phases due to their inability to mixing with the water fully and when used the DNA is pulled to the aqueous phase which formed. Is sometimes used ether saturated with water as it works to dissolve the phenol and chloroform before deposition of DNA aqueos phase as using cold alcohol, the presence of salt, such as sodium acetate for the purpose of DNA deposition. As the salt works on:

1 - Keep the secondary structure of DNA without denaturation.

2 - inhibits the growth of microorganisms.

3 - equivalent to the base of the SDS

3) Mechanical Treatments:

This processes by using Centrifuge. The type of treatments used in extraction depends on the type of tissue you want to isolate the DNA from it, and in general, the extraction of DNA from animal tissue is much easier than the isolation of plant tissue for hardness cellular walls plants add to oppose the presence of sugars and other metabolic product with purification processes.

Chromosomal DNA extraction from bacteria:

1) Grow bacteria in 3-4 ml of brain heart infusion broth for overnight at 37C°.

2) Centrifuge 1-1.5mL of bacterial culture in Eppendrof tube for 5min and remove the supernatant

3) Suspend sediment by 1.5mL of the lysis buffer consisting of:

400Mm Tris-Hcl, 60Mm EDTA, 150mM NaCl, 1% SDS PH = 8. And leave the mixture at room temperature for 10min to analyze the cell walls.

4) Add 150 µl of potassium acetate solution consisting of:.

5M potassium acetate 60mL

Glacial acetic acid 11.5mL D.W. 28.5mL

5) Shake the tubes by vortex 10sec.

6) Centrifuge for 1min\10000xg.

7) Transfer the upper aqueous phase to another tube and add a similar size of a mixture phenol: chloroform (1:5) at pH = 8 and shake well the contents of the tube.

8) Centrifuge for 1min\10000xg.

9) Transfer supernatant to another tube and add a similar volume of Isopropanol and shake well

10) Centrifuge for 2min\10000xg, and neglect the supernatant.

11) Wash the sediment by 1 mL of alcohol Ethanol 70% .

12) Centrifuge for 1min\10000xg, neglect the supernatant and

placed in an inverted on filter paper to dry completely.

13) Dissolve the sediment by)10-20 µl(from TE buffer and keep freezing (-20C°) Briefly, steps to DNA Extraction:

1. Break the cells to open and expose the DNA.

2. Remove membrane lipids by adding detergent.

3. Precipitate DNA with an alcohol — usually ethanol or Isopropanol. Since DNA is insoluble in these alcohols, it will aggregate together, giving a pellet upon centrifugation. This step also removes alcohol-soluble salt.

Extraction DNA by rapid boiling:

1) Grow bacteria overnight at 37C°,on Brain heart infusion agar

2) Suspend few colonies of bacteria in 1 mL distilled water on Eppendrof tube, and shake well by vortex.

3) Boil in a water bath for (10 min) and centrifuge directly , for 13000 rpm/5min.

4) separate the supernatant and put in another clean Eppendrof tube and then centrifuge tubes quickly (6000) rpm/ 2min, remove the supernatant and melts the pellet with a solution of the TE buffer.

Benefits of additives:.

1- TE buffer:

*** Disrupting the bacterial cell walls to contain EDTA, which works on:

* Helps to capture ions Mg++ & Ca++ from the cellular walls which provide stability of the cell wall.

* Stop the effectiveness of enzymes Nuclease such DNase (destroys the DNA).

* helps disengagement nuclear protein complex.

*** dissolving the DNA sample.

2- Sarkosyl or SDS: Ionic detergent works:.

* Denaturation proteins which acting on breaking peptide bonds and thus disengagement amino acids.

* Remove the fat which found in cell walls.

3- : Chloroform : Phenol

* Denaturation proteins.

* Eliminates the remaining phenol atoms in the DNA preparations (as phenol effect the solubility the DNA in water).

* Inhibit the effectiveness of the enzyme RNase.

4- Isopropanol: precipitation DNA.

5- Ethanol 70%: used to wash the DNA sample which helps to reduce dry sediment by alcohol .