Lab7 Molecular Genetic Third Step

B- Bacterial Transformation

Transformation mechanism includes genetic informations transfer through DNA piece releases from donor strain after its cell lysis , after that DNA piece will enter into the recipient strain , and thus emergence of new characters will be inherited by recipient cells .

Transformation phenomenon had been discovered in *Streptococcus pneumoniae* by scientist Griffith 1928 when he noticed the possibility of converting the rough form (R) of this bacterial colonies to the smooth form (S) *in vivo* , then he thought that it must be a factor responsible for this it was named Transformation Agent .

After that experiments by Macloed , McCarty and Avery 1944 proved the transformation agent is DNA which gave an evidence on the DNA is the genetic material . Transformation dose not occur easily in all bacterial species , recipient cells that allow the entry of DNA is called (Competent Cells) which are physiologically competent to accept the external DNA .

As examples on bacteria have the ability to transform *Bacillus subtilis* , *Haemophilus influenzae* , *E.coli* , *Rhizobium* ssp . and *S.pneumoniae* . Cells usually be competent at the end of log phase and the beginning of stationary phase , this dues to the response of quorum sensing . For example genes responsible for cell competence in *B.subtilis* will due to cell entry in early stage of sporulation , development of cell competence in this phase is not associate with nutritions decreasing only but with certain products accumulation are called (Competence Factors) which activate the gene expression of another genes necessary for competence , and the level of these factors is depending on cells concentration , competence develops only in stage of high cellular density which is associated with quorum sensing .

Cells can be transformed from non-competent to competent-cells *In-vitro* by the following methods :

a-Chemical Method : which includes treatment with one of the following materials :-

1- Cells treatment with Calcium shock : It is a process of cell incubation in cold $CaCl_2$ solution for along period , which works on pores formation on the recipient cells walls , it is used cold to avoid cells killing .

2- Cells treatment with Dimethyl Sulfoxide (DMSO) .

3- Addition of Polyethylene glycol to decrease negative charges on the cells surfaces .

b-Physical Method : which includes cells induction by cells exposure to high – voltage electrical impulses (Electroporation) , in spite of this there are important strains from industrial , environmental and serological point cannot be transformed to competent cells . While there are naturally competent cells such as *Neisseria* , *Haemophilus* and others , their acceptance to DNA molecules is depending on the presence of short sequence in competent cells DNA which pick up the DNA of the same species efficiently .

Researches show that transform agent is found in the extract free of cells (DNA only) participetate by alcohol and acetone , dose not affect by heating till 80c° but it loses its activity in boiling or enzymes , and there is a close relationship between purity of DNA and increase the effectiveness of the transformation .

Stage of Transformation Process :-

1-DNA – Binding Stage :- DNA fragment of donor strain will bind competent cell wall by specific receptors on its surface contribute to the DNA fragments will compete between each other for binding with these receptors , and then these DNA fragments will be fragments by endonuclease enzymes of the recipient cell but it able for acceptance of donor cell DNA .

2- DNA – Entry to Recepient Cell Stage :- DNA fragment will enter to the recipient cell as (single strand) this process needs to energy to enable DNA fragment from crossing the cell and reach membrane the cytoplasm .

3- After DNA- Entry to the Recipient Cells Stage :- After DNA fragment entry , this fragment begins to identify homologous genetic location on the recipient cell chromosome , after that genetic exchange will occur between donor cell DNA and recipient cell DNA , so recipient cell chromosome will contain genetic characters from donor cell .

- Some competent cells can pick up whole genome of some bacteriophages in phenomenon called Transfection .

There is an example on new characters transfer to the recipient cells by transformation mechanism , is antibiotic resistance development of *S.pneumoniae* which occurs by exchange a part of genes that encode for the target enzymes for penicillin by the similar DNA molecule from *Streptococcus* bacteria located in the mouth which is naturally resistant to this antibiotic .

Method:

1- Prepare broth culture of *S.aureus* incubated for 18 hr .

2- Heat the broth culture in 80c° for 5min to kill bacteria and avoid DNA denaturation .

3- Attend another broth culture of *S.epidermidis* , add to this culture cold $CaCl_2$ solution to work on loosening the cells walls.

4- Mix the two cultures and incubated for 30 min in 37c°.

5- When incubation period ends, take a drop from this mixture and culture it on blood agar medium by streaking to observe hemolysis .

- If there is no $CaCl_2$ addition , must use heating in 42c° and then incubate at the second time in 37c°.