Isolation of Spontaneous Mutation

Isolation of Antibiotic Resistance

All living organism including bacteria are suffering from exposure to spontaneous mutations which rarely or randomly occur , the most important reasons of their occurrence :-

1- U.V. light (10-400)nm especially wave length (260)nm .

2- Chemical Mutagens.

3- Error in DNA replication.

If the mutation is more suitable to the environmental conditions so its growth will overcome the mother culture , so it will be dominant , Antibiotic resistance spontaneous mutations can be observed easily when the bacteria grow with presence of antibiotic concentration which due to inhibition of normal microbes . It should be noted that spontaneous mutations arise from inadvertently in the use of mutagens .

Materials and Methods

1- Prepare *E.coli*   broth culture using nutrient broth incubated for (18-24) hrs.

2- Attend serial dilutions to 10^{-5} from broth culture by normal saline , take 0.1 ml from last dilution 10^{-5} and cultured in plate contains nutrient agar only by spreader incubated in 37c° for 24hrs .For viable count .

3- Apply the following law :

Viable Cell count / ml = Dilution factor x No. of colonies in 1ml.

$$= \text{Average no. of colonies in the plate} \times 10 \times 10^{5}$$

4- 0.1ml is taken from the stock culture and cultured by spreader in plate contains nutrient agar with 10μg/ml of antibiotic incubated in 37c° for 24hrs. (2 replicates) . read the results in the next day .

5- Apply the following law :

No. of Mutant bacteria = Average no.of mutant bacterial colonies in two plates x10

6- Apply the following law for getting spontaneous mutation frequency :

No. of mutant bacteria= Average no.of mutant bacteria in 1ml / viable cell count .