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Lab 6: BACTERIAL MUTATION

Mutation is a very important concept in biology today that leads to variations in genes. A mutation is a permanent alteration in the sequence of nitrogenous bases of a DNA molecule. The result of a mutation is generally a change in the end-product specified by that gene. In some cases, a mutation can be beneficial if a new metabolic activity arises in a microorganism, or it can be detrimental if a metabolic activity is lost. Mutations can be spontaneous, or induced by a mutagen in the environment.

The mutation is divided in general into two main categories:

1) Spontaneous mutation

2) Induced mutation

Spontaneous mutation: It is an auto mutation which occurs in the nature without participating the human and it occurs because of either physical or chemical reasons.

a) **Physical reasons**: It represents by displaying to different types of rays or change in temperature, pH, pressure or other physical effects.

b) **Chemical reasons:** It represents by displaying to the products of industry, like pesticide, stream, smock, food preservations…. etc.

Identifying these mutations requires detection methods. Classic methods i,e, Replica plating, gradient plate, Penicillin enrichment, Ames test, Use of chromogenic substrate and novel tests such as Polymerase chain reaction (PCR) and Gel electrophoresis, Gene probes and Southern blotting, DNA sequencing and DNA microarray are some of these methods that are highlighted in this laboratory

Gradient plate method for isolation the spontaneous mutation:

This method is considered as simple and easy for primary detection of spontaneous mutation.

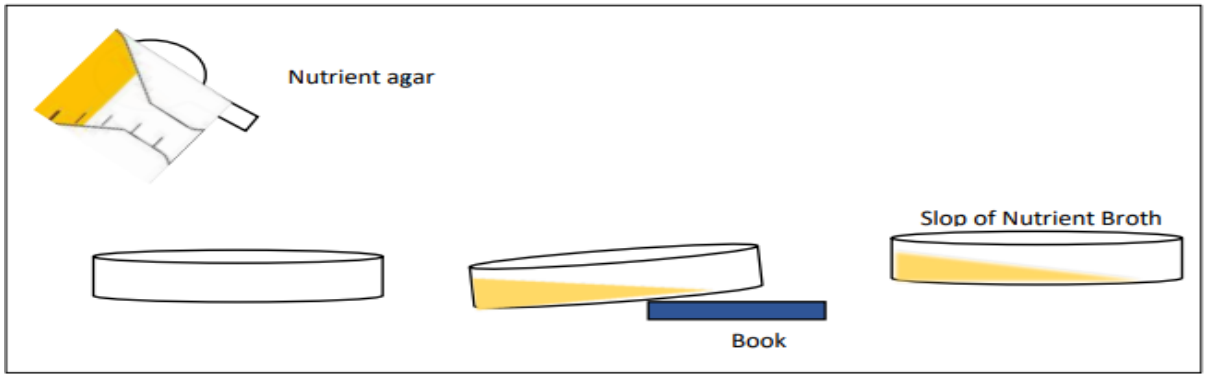
Procedure:

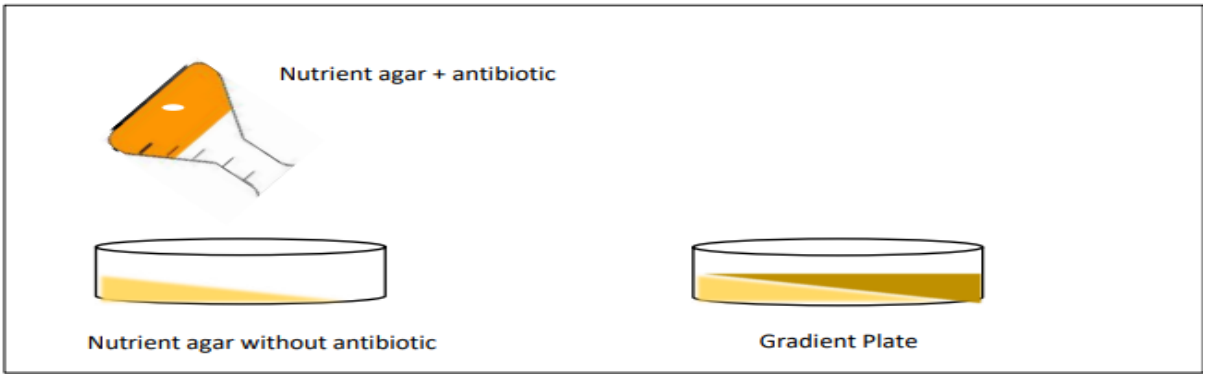
1) Prepare nutrient agar, sterile it and pour it into sterile plates as average 15 ml in each plate (half the plate), after that, the plates are slopped by using any book edge, leave it till it gets solidified.

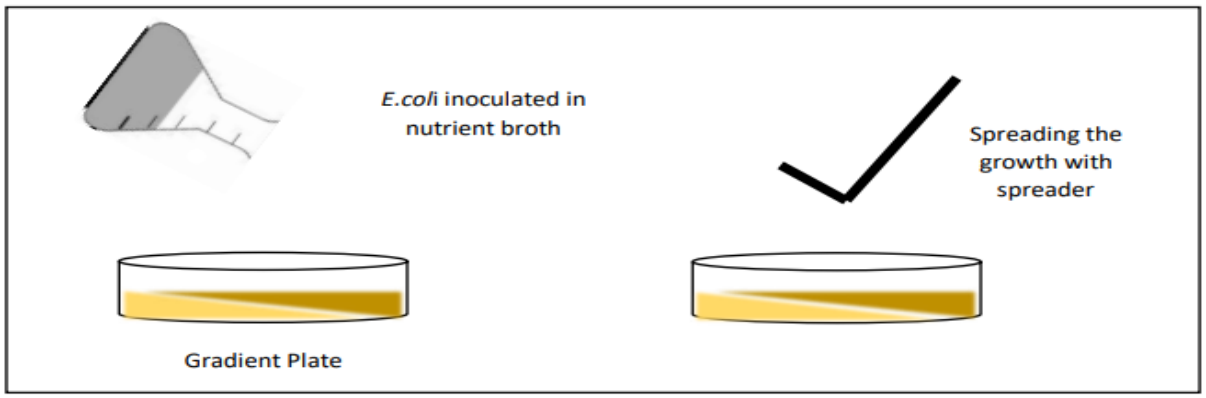
2) Prepare another nutrient agar, after sterilization cool it to 45 C. Add the chosen antibiotic for at concentration of 500 µg/ml to the media and move the flask in a circle way to mix the antibiotic properly with the media. (foam should be avoided).

3) The (nutrient agar + antibiotic) is poured over the previous solidified slopped nutrient agar until the plate is full and the media reaches to a straight line.

4) After the plates are solidified, inoculate the plates with E. coli by taking 0.2 ml from the original growth and spread it by using a spreader over the whole surface of the media, then incubate the plates for 24 hrs. at 37 C.







Discussion the results:

1) If there is no growth; this means that the bacteria do not have the ability to resist the antibiotic in this concentration.

2) If there is full growth; this means that bacteria have the ability to resist the antibiotic.

3) If the growth appears only in the region of low concentration of antibiotic in the plate, this means that the bacteria can only resist the low concentration of this antibiotic.

• In this case loop is used to touch single colony and move it towards the high concentration region of antibiotics.

• Incubate the plate for 24 hrs., then if there is any growth, the colony is transferred again to the high concentration region of the palate; then watch the results:

A) If there is no further growth, this mutation is called (single mutation)

Single Step Mutation: Is a mutation in which the bacteria can resist limited concentration of the antibiotic but they can not be grown on higher concentration.

B) If there is any further growth, this mutation is called (Multi Step Mutation)

Multi Step mutation: Is a mutation in which the bacteria can be grown in a higher concentration than the original concentrations in a gradient way.