Identifying Mutants

With bacteria mutants can be identified more easily, because bacteria produce very large populations very quickly.

Mutant can be identify by 2 methods:

1-Positive (direct) selection involves the selection of mutant cells and rejection of non mutant cells. For example: plating out bacteria on a medium containing penicillin. Survivors, which are penicillin - resistant mutants, can be isolated.

2- Negative (indirect) selectiont or replica plating: This technique has been discovered by **Esther Lederberg & her husband, Joshua Lederberg** in1951. (Figure 1), is used for detect, for example, auxotrophs that have nutritional requirements not possessed by the parent (non mutant) cell. To isolate auxotrophs, colonies growing on a master plate containing a complete medium can be transferred by a sterile velvet pad is pressed onto the master plate, and the colonies are transferred simultaneously to a minimal medium, which lacks essential nutrients such as the required amino acid. An auxotrophic mutant will fail to appear on the minimal medium.

Figure 1: Right,**Esther Lederberg**(1922-2006) was a major pioneer of bacterial genetics. She discovered the lambda phage(a bacterial virus which is widely used as a tool to study gene regulation and genetic recombination). She also invented the replica plating techniqu(which is used to isolate and analyse bacterial mutants and track antibiotic resistance).

Left**,Joshua Lederberg** (1925 – 2008) was an American molecular biologist known for his work in microbial genetics, artificial intelligence, and the United States space program. He was 33 years old when he won the 1958 Nobel Prize in Physiology or Medicine for discovering that bacteria can mate and exchange genes (bacterial conjugation). He shared the prize with Edward Tatum and George Beadle, who won for their work with genetics.
In addition to his contributions to biology, Lederberg did extensive research in artificial intelligence. This included work in the NASA experimental programs seeking life on Mars.

Principle

Replica plating involves creation of exact copy of master plate(exact number and exact location of each colony). The exact copy of master plate can be created with the help of sterile velvet leather, cotton or chamois leather stamp or clothes. These clothes or stamp acts as microneedle for inoculation of microbes when pressed against master plate bacterial culture. Impressing a petri plate on to the velveteen. Organisms will be transferred from their positions on the fabric pile to the nutrient medium in the plate.then we can transfer these colonies to another media (plate) to investigate mutants(Figure 2).

Materials

Sterile velvet

Master plate(isolated colocies of *Salmonella typhimurium* growing on Loria agar contain histidine.

plate of Loria agar or nutrient agar with histidine (control plate: which ia an exact copy of the master plate)

plate of Loria agar or nutrient agar without histidine (test plate :by wich you will check the presence(+) or absence(-) the mutant .

Procedure

1-Mount a piece of sterile velvet by stretching it on a cylindrical metallic block (slightly smaller than Petri dish).

2-Place the block with velvet side facing upwards.

3-Invert the master plate with bacterial cells growing on media containing histidine. and gently press against the velvet. The number of projecting fibres of the velvet (almost 1000/sq. inch) act as inoculating needles sampling every clone of the cells on the agar.

4-Remove the master plate.

5- Now take new plate of Loria agar with histidine (control plate) and place it inverted on the stamp.press it gently and then remove it.

6- Repeat step5 using new plate of Loria agar without histidine (test plate)

7-Incubate the plates in incubator 37°C for 24hr.

8-Examine the number and location of each growing colony and copare them to the colonies on the master plate.

9-colony that appears on the control plate(a copy of master plate) and disappear from the test plate represent mutant (auxotrogh) bacteria and the replica test is positive(+).(Figure 3).

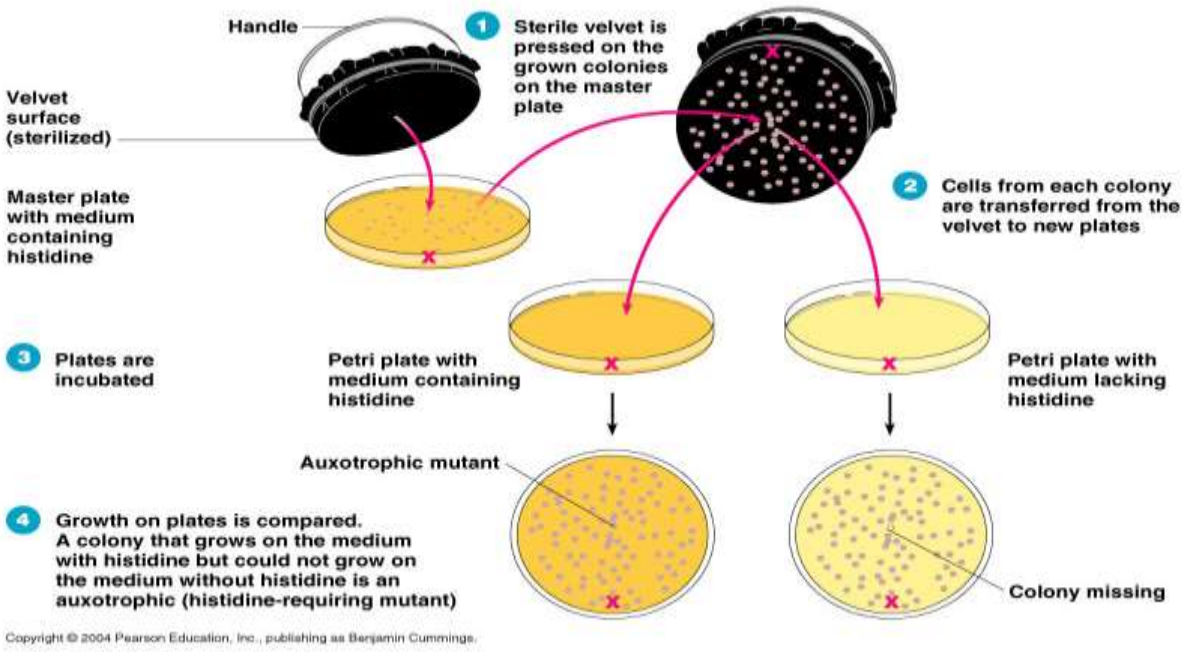

Figure 2: Replica plating technique .

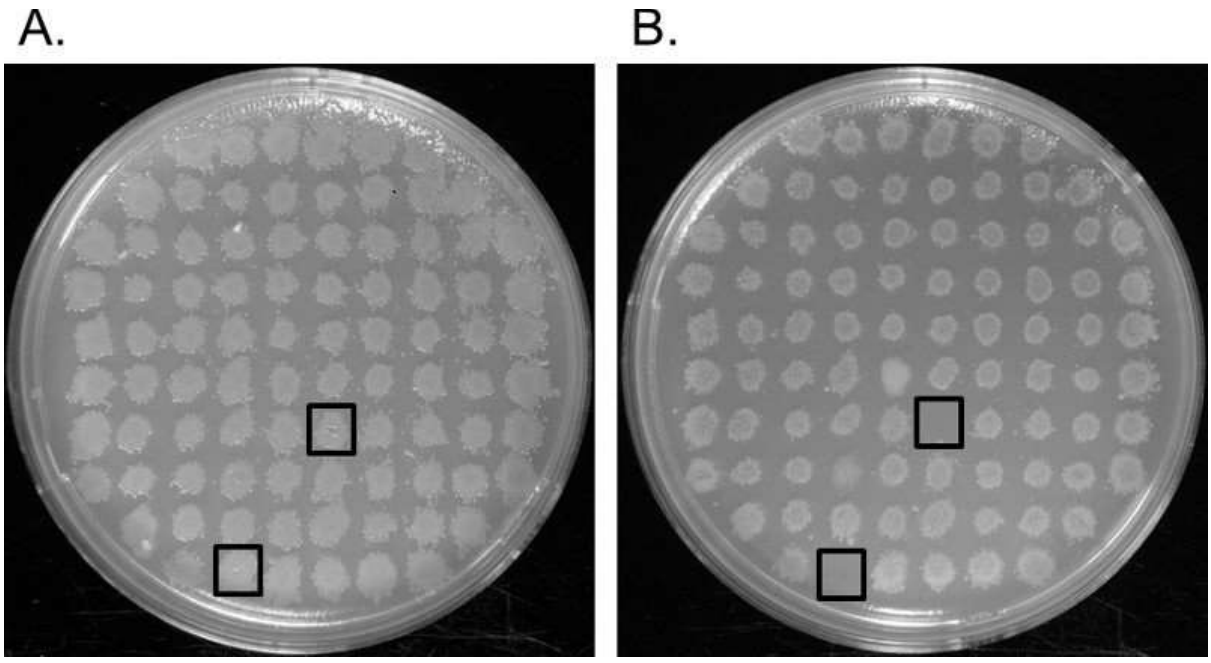

Figure 3: Reading the resultes of Replica plating technique .A: Control plate(Replica test negative). B: Test plate (Replica test positive).