Detection of auxotrophic mutations

Auxotrophic Mutant

- Auxotrophic mutants are strains that require a growth supplement that the organism isolated from nature (the wild-type strain) does not require.
- Auxotrophic mutants are unable to synthesize some biochemical essential for their growth and development that wild-type cells are capable of producing.
- Such mutants cannot be detected using the simple approach. These mutants are detected by Replica Plating method.

Replica Plating Method:

If an organism has the ability to produce mutant strains resistant to antibiotics, the nature of mutation, whether it is spontaneous or induced have to be tested. It would be a difficult task to identify a few mutant colonies from a vast population of 100-500 colonies. This can be accomplished by a replica plating technique. The technique was developed by Joshua and Esther Lederberg in 1952 for providing the direct evidence for the existence of pre-existing mutations. This technique isolates both nutritional mutants and antibiotic resistant mutants. Their actual experiment concerned with replicating master plates of sensitive cells to two or more plates containing either streptomycin or T1phage.

Replica plating allows the observation of microbes under a series of growth conditions. The bacteria are grown in an environment that is not selective for given mutation. This technique is used to transfer the members of each colony to a selective environment. A simple velveteen covered colony transfer device is used to transfer the colonies in nutrient agar medium supplemented with or without a particular antibiotic or nutrient. The fibers of velvet act as fine inoculating needles, picking up the bacterial cells from the surface of this master plate. The velvet with its attached microbes is then touched to the surface of a sterile agar plate, inoculating it. In this manner, microbes can be repeatedly stamped onto media of differing composition. By comparing the presence of colonies following incubation we can indirectly determine the mutant colonies by their absence in the selective environment. A colony that develops on a complete medium fail to develop on a minimal medium that lacks a specific growth factor, the occurrence of a nutritional mutant is indicated. The microbes that do not grow on the minimal medium represent auxotrophic strains. A simple velveteen covered colony transfer device is used to transfer the colonies in nutrient agar medium supplemented with or without a particular antibiotic or nutrient. A colony

that develops on a complete medium fail to develop on a minimal medium that lacks a specific growth factor, the occurrence of a nutritional mutant is indicated. The microbes that do not grow on the minimal medium represent auxotrophic strains. This method has been applied in numerous experiments to identify the occurrence of mutations. Many of the biochemical pathways in microbes were elucidated in this way by using nutritional mutants (Fig 1).

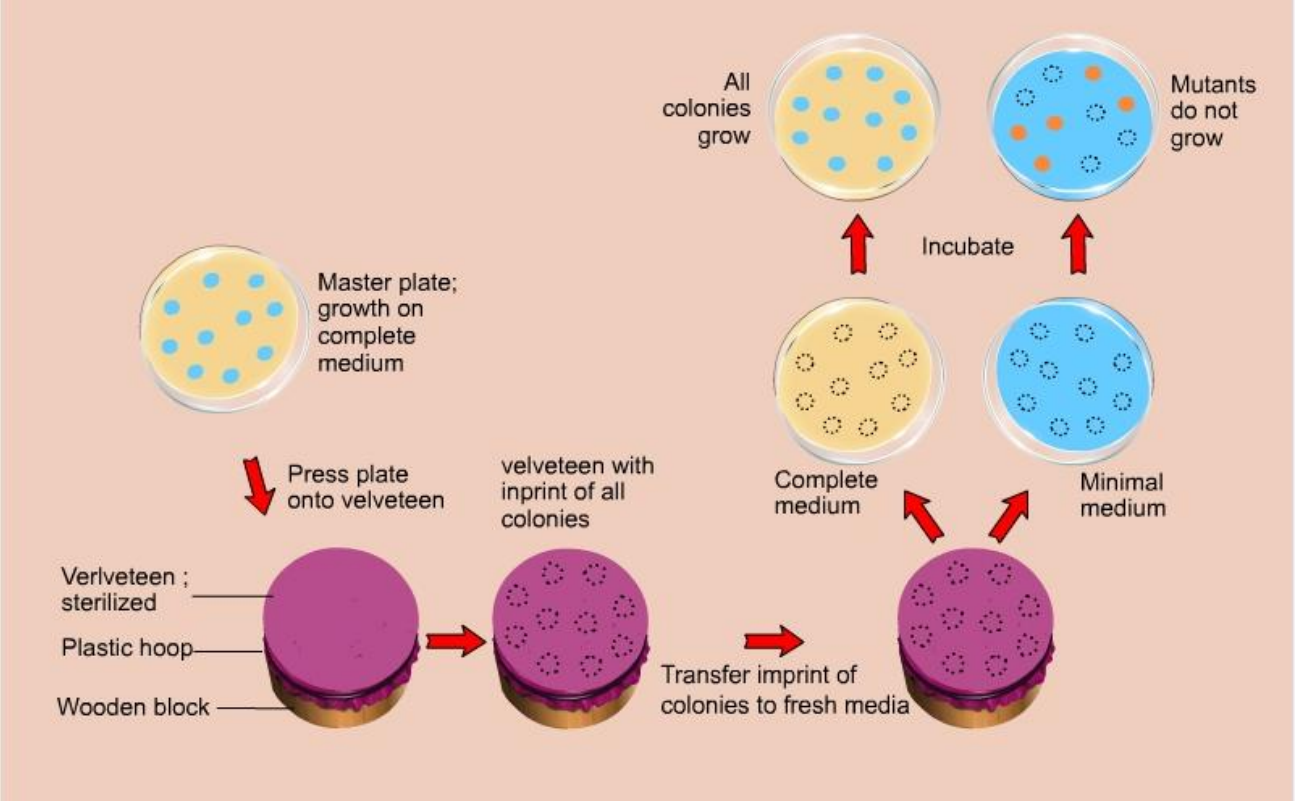

Fig 1: Replica plating technique

Ames test

Ames test it is a biological assay to assess the mutagenic potential of chemical compounds. It utilizes bacteria to test whether a given chemical can cause mutations in the DNA of the test organism. The test was developed by Bruce N. Ames in 1970s to determine if a chemical at hand is a mutagen.

1. Ames test uses several strains of bacteria (*Salmonella, E.coli*) that carry a particular mutation.
2. Point mutations are made in the histidine (Salmonella typhimurium) or the tryptophan (Escherichia coli) operon, rendering the bacteria incapable of producing the corresponding amino acid.
3. These mutations result in his- or trp- organisms that cannot grow unless histidine or tryptophan is supplied.

4. But culturing His- *Salmonella* is in a media containing certain chemicals, causes mutation in histidine encoding gene, such that they regain the ability to synthesize histidine **(His+**). This is to say that when a mutagenic event occurs, base substitutions or frameshifts within the gene can cause a reversion to amino acid prototrophy. This is the reverse mutation.
5. These reverted bacteria will then grow in histidine- or tryptophan-deficient media, respectively.
6. The frequency of cell forming colonies gives the frequency of reversion. The frequency of spontaneous reversion to his^+ is quite rare i.e. 10^{-8}
7. Ames test is routinely used to investigate the mutagenicity of various chemicals. Some of the chemicals may become mutagenic only when they acted upon liver enzymes. Eg. Nitrate themselves are neither mutagenic nor carcinogenic, but in eukaryotic cells, nitrates are converted to nitrosamines, which are highly mutagenic and carcinogenic.

A sample's mutagenic potential is assessed by exposing amino acid-requiring organisms to varying concentrations of chemical and selecting for the reversion event. Media lacking the specific amino acid are used for this selection which allow only those cells that have undergone the reversion to histidine / tryptophan prototrophy to survive and grow. If the test sample causes this reversion, it is a mutagen.

Procedure: -

- The his^- bacterial cells are incubated with the liver extract and then plated onto a medium containing trace of histidine, this serves as control plate.
- The test plates contain the same medium but the his^- cells are not treated with liver extract.
- The test chemical is treated with the rat liver extract and a filter paper disk is soaked in this solution.
- The filter paper disc is placed onto the medium of test plate. The chemical present in the filter paper acts on the his^- cells growing in the test plate.
- The frequency of colonies formed in the control plate and the test plate are compared and an increase in frequency of the test plate indicates the test chemical to be mutagenic.

- The control plate provides an estimate of the frequency of spontaneous reversion of the tester strain.
- The experimental plate shows the frequency of reversion induced by the test chemical.

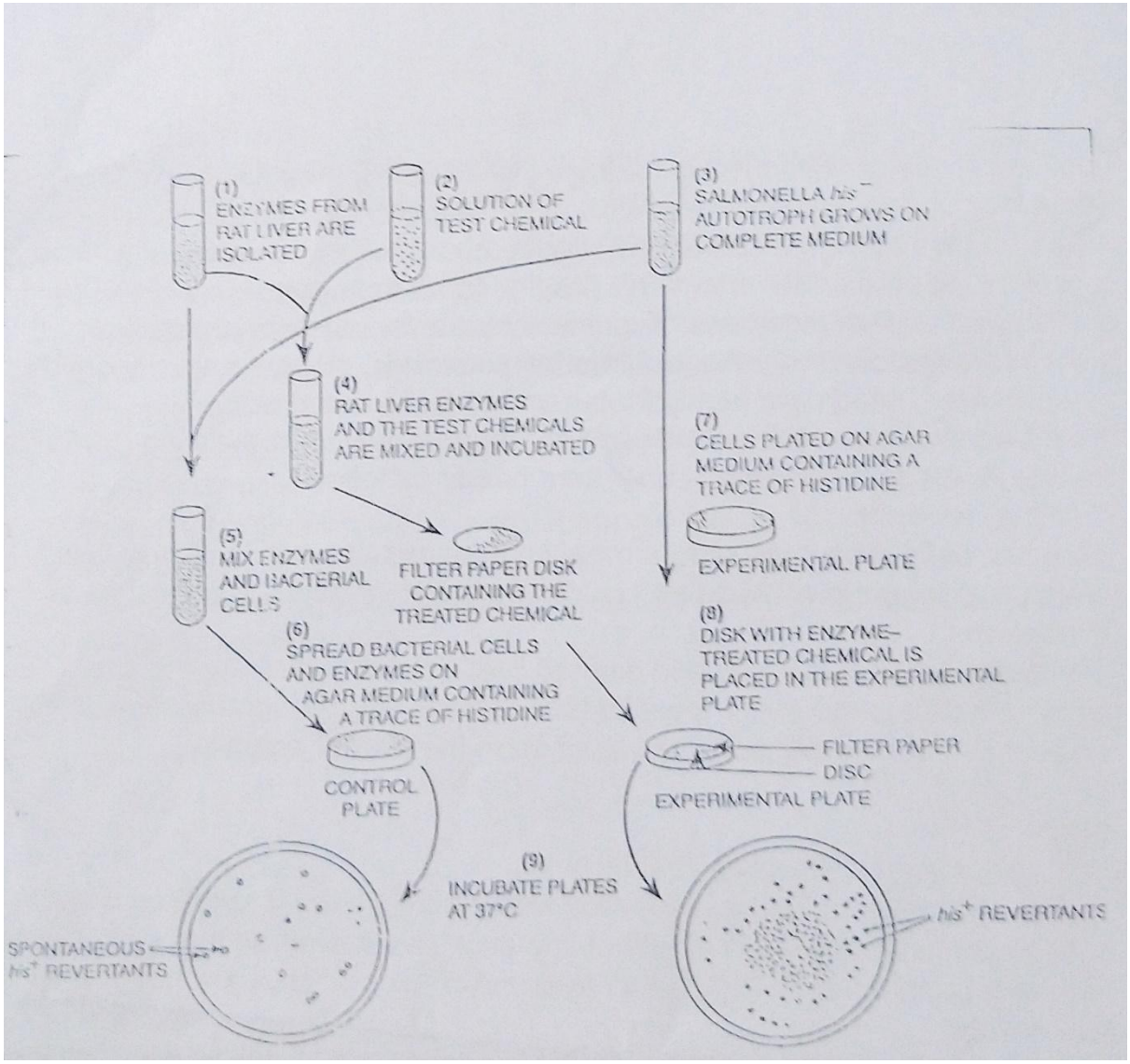

Fig. 2 Ames test

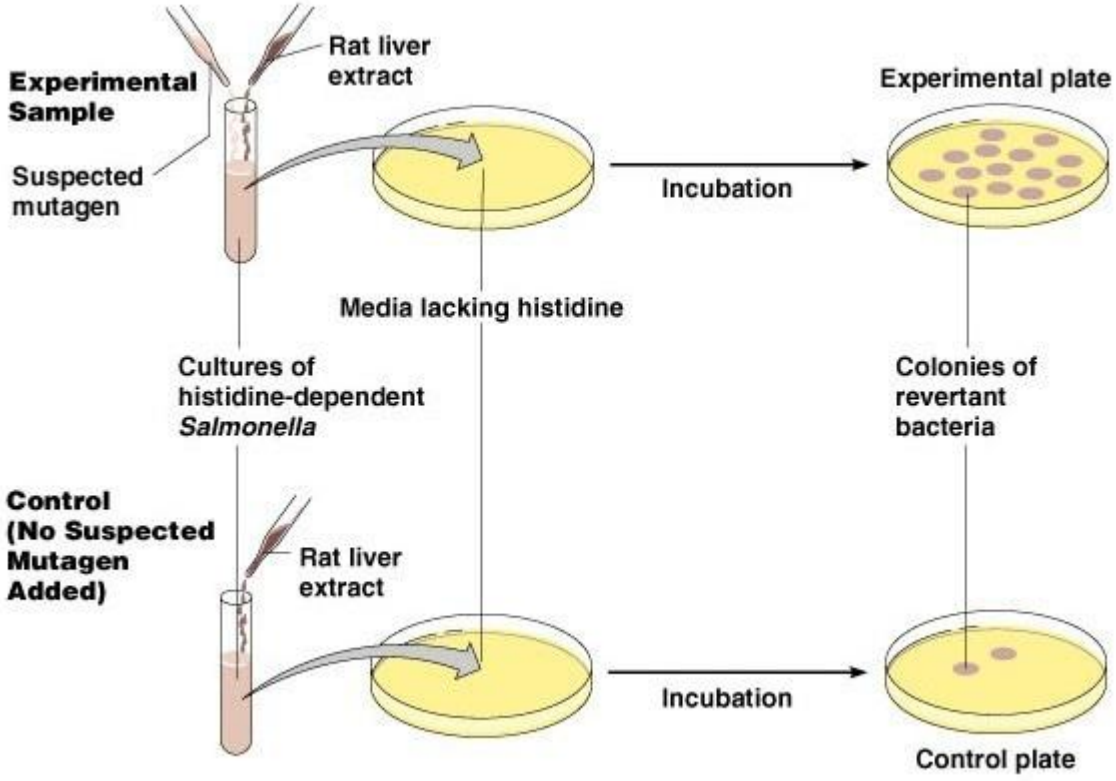

Fig. 3 Ames test

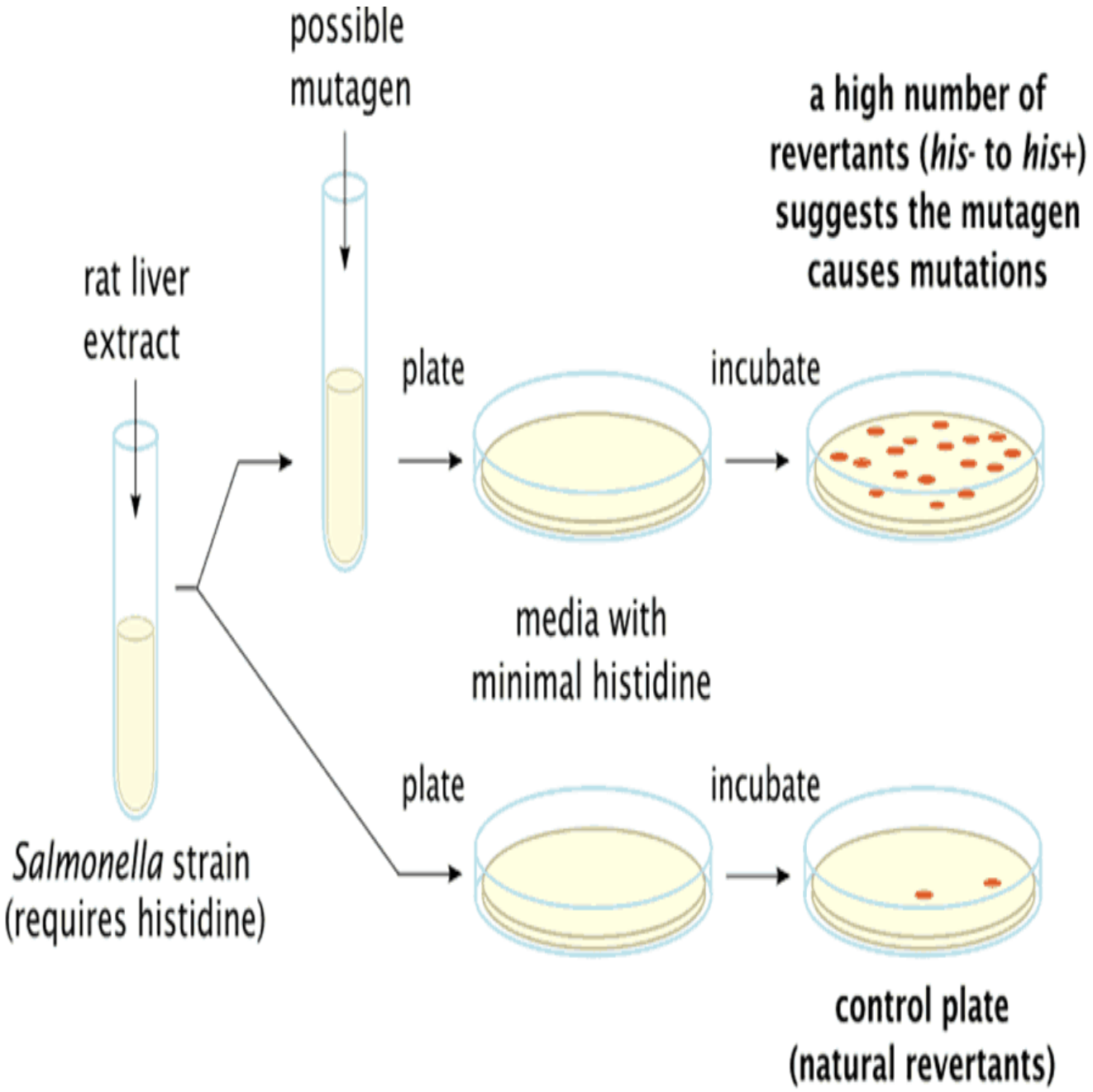

Fig. 4 Ames test