Welcome students, my name is Ms. Dviti Mapari, Assistant Professor, Department

of Microbiology, Government College, Khandola. So today I will be taking the module titled principle,methodology and significance of replica plating.

The outline is as follows : Principle of replica plating, Methodology of replica plating and Significance of replica plating.

The students will be able to learn to perform replica plating as well as will be able to learn the applications of replica plating.

Let us start with replica plating.

The general method of replica plating was described by Joshua Lederberg and Esther M. Lederberg in 1952.

Replica plating method is used to detect auxotrophic mutants.
It distinguishes between mutants and wild type strain based on their ability to grow in the absence of a particular biosynthetic end product.

Now let us see what the mutants are and what a wild type strain is.
Auxotrophs a group of microorganisms that have lost the ability to synthesize a certain substance that is required for their growth. Owing to the presence of mutation so an auxotrophic mutant is an organism which has undergone a mutation and thus has lost the ability to synthesize a certain substance that is required for their growth due to mutation.

What is a wild type organismPrototroph ? Protrotroph is the naturally existing o organism which has not undergone any mutation. So the replica plating method again in the principle I have mentioned is used to detect aurotrophic auxotrophic mutants. It distinguishes between the mutants and the wild type and based on their ability to grow in the absence of a particular biosynthetic end product.

The velveteen cloth that is used in this procedure. The fibers of this Velveteen cloth act as atiny inoculating needles which helps in sampling all the colonies in one o operation.

So here I have given an example of a lysinee auxotrophauxotroph. Lysine auxotroph auxotrophwill growin lysine in esupplemented media only, but not on a medium lacking an adequate supply of lysine elysine because the oorganism cannot s synthesize lysineit has lost the ability due to mutation.
There are three types of media here. Minimal media, minimal media supplemented with lysine and the minimal media s supplemented with arginine. So when the auxotroph is prototroph plated onto a minimal media it is not growing as compared to the other plate where it has a minimal medium supplemented with lysine. The lysine auxotroph is growing on lysine supplemented media .The auxotroph is not growing

, in a minimal media supplemented with arginine. That means the lysine auxotroph needs lysine to be supplemented in the medium for its growth to occur.
Organism not undergone any mutation, so all the biosynthetic pathway to produce lysine are being functional as a result of which it is growing on all the plates.

Now let us see the protocol of replica plating. Here first the mutants are generated by treating a bacterial culture with a mutagen. Mutagens are the mutation causing agent. The culture containing wildtype and auxotroph is plated on to a complete medium. After the colonies have developed after certain period of incubation a piece of sterile velveteen cloth where its fibers are acting as a inoculating needles,this sterile velveteen cloth is pressed onto the plates
of face to pick up all colonies from the plate in one operation.The velvet is pressed onto the surface of other plates, that is, the one plate containing complete medium and one containing medium without the required supplement that is missing from the the plates. Incubated for a required period.On the next day we have to determine the location of the colony on the both the medium.Here the auxotroph will grow on the complete media, but not on the media, lacking the
supplement. So you can identify the auxotroph onto the medium and does the exact colony can be picked up from the complete medium and can be cultured.
Now let us see how replica plating works. This involves the protocol in the schematic form.
In this test you here, you'll have *E.coli* suspension,which is grown at 24hrs which is incubated an grown to its maximum potential. Then this suspension is treated with a mutagen such ,nitrosoguanidie. After the *E. Coli* has been treated with the mutation causing agent. It is
allowed for some time to set up, so that mutations can occur in the *E. Coli* culture.
After that the bacteria that is the *E. Coli* is being inoculated onto a complete medium, so this plate is the complete medium complete media. It is the.
Medium in which there is minimalmedia as well as it is supplemented with the lysine.
After spread plating onto this plate, this plate is further incubated. After incubation, we notice that both wild type as well as the mutant survivors from the mutant survivor colonies are produced onto the plate. Because this is a complete medium. So this is a master played. Another important thing, the arrangement of the
plate is very very important as a result of which this cross mark has been made onto the plates to keep it a line. Next, the replica block having a sterilized velvet surface is being taken. This replica block is stamped onto the plate that is the master plate picking up all the colonies. Here the colonies could be survival mutant survivors as well as it could be wild colonies in this plate.
In the same alignment, this already stamped replica block is taken and it is stamped onto 2 plates. The first plate is the complete medium containing the lysine like that of master plate and the second plate is a petri plate containing medium lacking lysine.
So here this both the plates.After stamping this colonies here this is the imprint of the colonies which have been picked up from your can be seen here.This being impinged and imprinted on this two plates. Then both the plates are allowed to incubate further and after incubation you observe this particular observation.

Identify the auxotroph as colony growing on complete media but not on medium lacking lysine .So here in this place that is the complete medium containing lysine, all the colonies are growing at that particular location as that of the master
plate. Where as in the medium lacking the lysine you can see that if you compare this, both the plates with that of the master plate as well, you can see that your there's no colony at that particular location, so determining the location of the colonies as well as maintaining the alignment of the replicablock with that of the petri
plates is very, very important. So here you can see that since this medium is lacking lysine a result of which the colony is not grown here. That means this colony which is here is a lysine auxotroph , which is growing on a medium containing lysine. But it is not growing on a medium that is lacking license. So you can identify this colony at this particular location as a lysine auxotroph and you can pick up this colony an inoculate this auxotroph colony into a complete media .

So that was the protocol of replica plating.

Now here are the applications of replica plating.
Replica plating is is used in the classification of colonies differing from each other in a number of non nutritional requirements as well as they are being used in the isolation of new mutants streams. This technique and its variations
have found wide use in microbiology and have been successfully applied to bacteria like actinomycetes as well as to unicellular algae.

These are the references that have been used
for this particular presentation . Thank you.