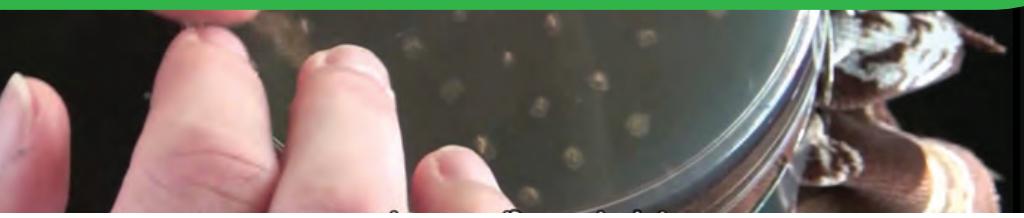
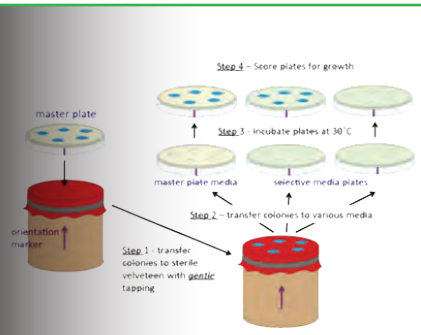
REPLICA PLATING





1. Aim

To study the technique of replica plating for identificationof *E coli* auxotrophic mutants.

2. Introduction

Replica plating is a technique in which one or more secondary Petri plates containing different solid (agar-based) selective growth media (lacking nutrients or containing chemical growth inhibitors such as antibiotics) are inoculated with the same colonies of microorganisms from a primary plate (or master plate), reproducing the original spatial pattern of colonies. The technique involves pressing a velveteen-covered disk, and then imprinting secondary plates with cells in colonies obtained from the original plate. Generally, large numbers of colonies (roughly 30-300) are replica plated due to the difficulty in streaking each out individually onto a separate plate.

The purpose of replica plating is to be able to compare the master plate and any secondary plates, typically to screen for a desired phenotype. For example, when a colony that was present on the primary plate (or master plate), fails to appear on a secondary plate, it shows that the colony was sensitive to a substance on that particular secondary plate. Common screenable phenotypes include auxotrophic and antibiotic resistance.

Replica plating is especially useful for "negative screening". For example, if one wanted to select colonies that were sensitive to ampicillin, the primary plate could be replica plated on a secondary Amp^+ agar plate. The sensitive colonies on the secondary plate would die but the colonies could still be deduced from the primary plate since the two have the same spatial patterns from ampicillin resistant colonies. The sensitive colonies could then be picked off from the primary plate. A non-selective plate should be replica plated after the Amp^+ plate to confirm that the absence of growth on the selective plate is due to the selection itself and not because of a problem with transferring cells. If one sees growth on the third (non-selective) plate but not the second one, the selective agent is responsible for the lack of growth. If the non-selective plate shows no growth, one cannot say whether viable cells were transferred at all, and no conclusions can be made about the presence or absence of growth on selective media. This is particularly useful if there are questions about the age or viability of the cells on the original plate.

3. Materials Required

3.1. Biological Material: Primary cultures of *E. coli* XL-1 blue

3.2. Chemicals/Reagents: Luria Broth media (LB), LB-agar media, minimal media (Solution A and Solution B), amino acid solutions (1mg/mL) each of leucine, lysine, tryptophan, histidine; ampicillin solution (50 µg/mL).

3.3. Equipment: Laminar flow hood, spectrophotometer, shaker incubator, static incubator.

3.4. Glassware/Plastic ware: Autoclaved petri-plates, conical flasks, velvet cloth, micropipettes, toothpicks.

4. Procedure

i. A primary culture of the *E. coli* XL-1 Blue strain was setup by inoculating 1% v/v from an overnight grown *E. coli* culture.

ii. When the O.D of the culture became 0.6-0.7 at 600 nm, 200 µL of the culture was taken in 8 centrifuge tubes labeled as A1to A8 and similarly 200 µL of culture was taken in 8 centrifuge tubes labeled as B1 to B8.

iii. The tubes labeled A were exposed to UV radiation for 30mins and the tubes labelled B were exposed to UV for 20 mins.

iv. Two plates with LB agar were marked A and B respectively and divided into 8 sections A1 to A8, and B1 to B8.

v. The bacterial culture from each centrifuge tube was streaked on the corresponding section on the plate, using an autoclaved toothpick.

vi. The plates were left overnight at 37 ºC.

vii. Two plates with LB agar were marked A and B respectively and divided into 5 × 5 grids with cells labeled A1 to A25.

viii. Single colonies from A1, A2 etc were picked and placed on the corresponding cell in the grid.

ix. The same was repeated on the second plate.

x. Incubate overnight at 37 ºC.

xi. Colonies were obtained on each cell the next day. These plates are called master plates.

xii. 6 types of minimal media plates were prepared:

   a. Control: Only minimal media
   b. Leu^+: minimal media + leucine
   c. His^+: minimal media + histidine
   d. Lys^+: minimal media + lysine
   e. Trp^+: minimal media + tryptophan
   f. Amp^+: minimal media + ampicillin

xiii. Using an autoclaved velvet cloth wrapped on the base of a conical flask as a stamp, a replica of the master plate was picked up and stamped on all 6 minimal media plates.

xiv. The plates were incubated overnight at 37 ºC.

xv. The plates were observed for mutants the next day.

5. Observations

Fig. 1: Creating a master plate using a modified metal stamp and a 96 well plate

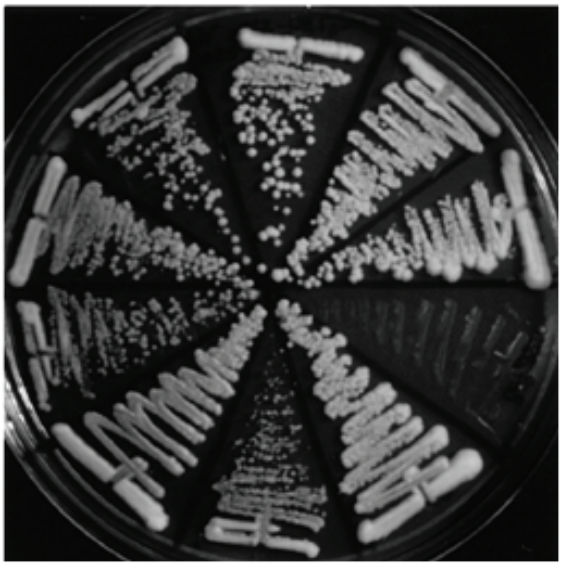


Fig. 2: Colonies grown after UV irradiation (Patch UV irradiated samples on Complete Media)

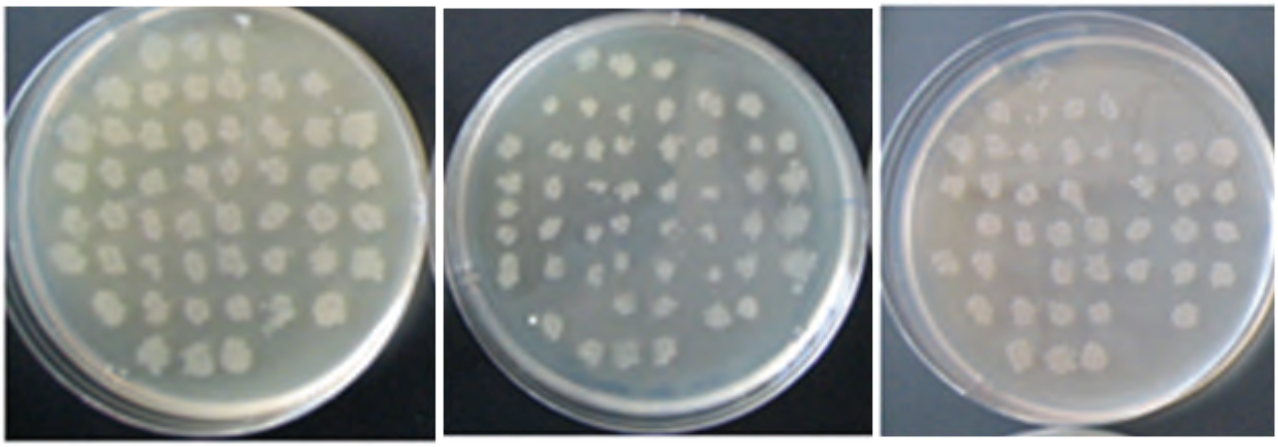


Fig. 3: A) The master plate, B) Replica on minimal media Leu+ C) Replica on minimal media His+

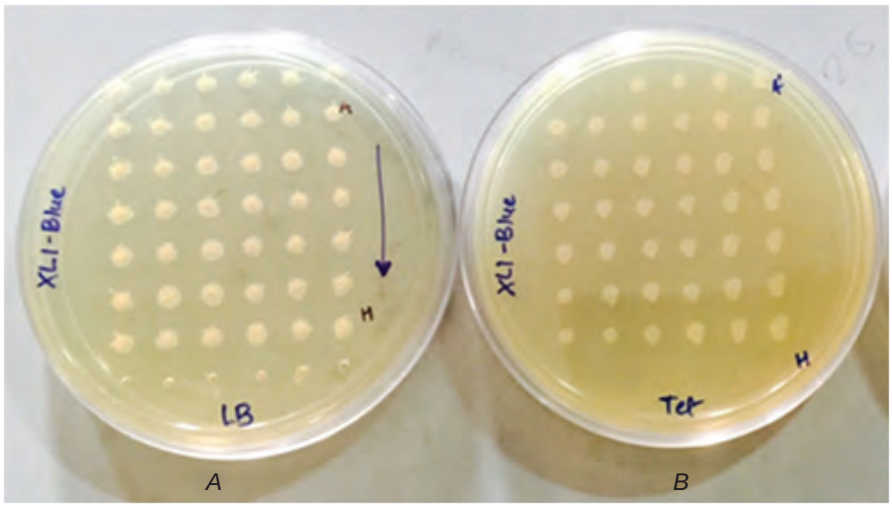


Fig. 4: Colonies of XL-1 Blue on A) Master plate, B) Replica on Tet+

6. Result

The technique of replica plating was performed.

7. Precautions

i. Perform all steps under aseptic conditions.

ii. The velvet side of the cloth should be pressed against the master plate as the fibers act as inoculation needle.

iii. Care must be taken to avoid exposure to UV radiations.

Suggested Reading(s)

i. Cappuccino, J., & Sherman, N. (2010). *Microbiology: A laboratory manual.* (9th ed.). Pearson Education Limited.

ii. Wiley, J. M., Sherwood, L. M., & Woolverton, C. J. (2013) *Prescott' Microbiology.* (9th ed.). McGraw Hill International.

iii. Atlas, R. M. (1997). *Principles of Microbiology.* (2nd ed.). WM. T. Brown Publishers. .

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