Plating

Introduction

The plating protocol is used to isolate, cultivate, and quantify microorganisms by transferring microbial samples onto agar media under aseptic conditions. Individual cells grow into visible colonies that can be used for identification, enumeration, and further microbiological analysis.

Reference:
1. Sanders, E. R. (2012). Aseptic laboratory techniques: plating methods. *Journal of Visualized Experiments (JoVE)*, (63), e3064.

Materials

- LB agar plate
- Antibiotic
- Toothpick

Procedure

Plate preperation

1. Mix DW (RO), Tryptone peptone NaCl, bacterial agar, NaOH(10N), Yeast extract in 500ml bottle.
2. Autoclave 500ml bottle
3. Add an appropriate antibiotic concentration .
4. Under sterile conditions, Pour plates (20mL/plate) and let plates solidify at room temperature (RT) for a few hours.
5. store it in the 4°C room.

Streak-Plating

6. Ensure the agar plate is pre-warmed to room temperature and free of surface condensation.
7. using flat toothpick lightly touch the culture.
8. Spread the inoculum across the first quadrant using a rapid, smooth, back-and-forth motion from the rim toward the center of the plate at a 10°-20° angle
9. Rotate the plate 90°

10. Use new toothpick, Pass the tool through the end of the streaks in the first quadrant, and streak back and forth into the empty second quadrant without returning to the first quadrant.

11. repeat step 9 and 10 twice

Incubation

12. Invert the Petri dish and incubate upside down to prevent condensation from dripping onto developing colonies.