Plaque Drop Assay

Introduction

With this assay you can determine the phage titer concentration

Materials

- sterile MYPGP Agar plate
- sterile dd. H_2O - 30 mL
- BHI Media - 20 mL to dissolve the phages
  - BHI Powder 37 g/L
  - Yeast Extract 3 g/L
- BHI Top agar - 20 mL
  - BHI Powder 37 g/L
  - Yeast Extract 3 g/L
  - 0.7 % Agar

Procedure

- Day 1

1. Take one row of the bacteria lawn from a fresh (max. 2 days old) *P.larvae* streak plate and dissolve them in 200 µL sterile dd.H_2O
2. Dissolve the bacteria (resuspend, vortex)
3. Plate out the 200 µL bacteria suspension onto a MYPGP agar plate
4. Incubate overnight at 37 °C

- Day 2

1. Prepare and autoclave BHI Top Agar and cool it at 55 °C
2. Turn the photometer on
3. warm the MYPGP Plates at 37 °C

4. Resuspend the *P.larvae* ONC Plate with 2 mL ddH_2O (sterile) and pipette it in a 1.5 mL reaction tube

5. Centrifuge 5,000 x g, 5 min in the eppifuge

6. Discard the supernatant, and dissolve the Pellet in ca. 250 µL ddH_2O (sterile)

7. Create 1:50 and 1:500 dilutions from the Probe and measure OD_{600}

8. Set the OD_{600} of the bacterial probe to be OD_{600} = 15

9. Pipette 50 µL OD_{600} = 15 bacterial suspension into a glass eprouvette, add 4 mL BHI-Top agar (~55 °C), mix, pour it quickly on a MYPGP agar plate
   - the plate should look smooth (no air bubbles)

10. Cool the MYPGP Plate at RT for 10 min

11. Prepare the phage dilutions (dilute in BHI media)
    - Dilutions from 10^-1 to 10 ^-9

12. Pipette a 5 µL drop from the phage dilution on the cooled MYPGP-Top agar plate

13. Let it sit at RT for 20-30 min

14. Incubate overnight at 37 °C, do not invert the plate

- Day 3

15. Count the plaques

16. Determine the Phage titer

- PFU/mL = (Plaque Count * 200) / Dilution