Phage TOP-agar experiments TU Delft

Aim:

With this experiment we want to compare the plaque size and the appearance of the plaque. Virulent strains or lytic lead to more clear plaques, thus the virulence of different phages can be compared with this experiment. In addition we can compare the speed that virions diffuse as this is indicated by the plaque size.

Equipment

- 0.6% top agar
- LB agar plates
- 37°C stove
- 37°C shaker
- BL21(DE3)
- T7 wild type
- Phage buffer (1x PBS, 1mM MgCl2, 1mM MgSO4)

Protocol

Day 1:

- Inoculate BL21(DE3) in fresh medium for an overnight culture at 37°C 150 rpm.

Day 2:

- Ten times serial dilute the T7 wildtype phage stock until 10^1, 10^2 and 10^3 pfu/ml.
- Aliquot 3 times 3 ml of liquid 0.6% top agar in 25 ml tubes, keep them in hot water to prevent the top agar from solidifying.
- Add 300µl of fresh BL21(DE3) overnight culture to the top agar (make sure the agar is not too hot). Immediately add 100µl of phage dilution and pour it over the LB agar plates.
- Incubate the plates at RT for 20 minutes.
- Incubate the plates at 37°C for 2 hours
- Count and measure plaques size
- Measure plaque size.
    - After around 2 hours plaques will appear, from then onwards measure every 30 minutes and take a picture (Exact plaque formation time may vary for different phages).