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🌐 Full plate & spot test plaque assays + PFU/mL calc. - aerobic bacteria

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FOOD Micro UCPH

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Protocol for plaque assays - either a full plate plaque assay that are more laborious but with high accuracy, or a spot test plaque assay that are for higher throughput but with less accuracy.

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Materials needed: Broth, agar plates, soft agar, bacterial culture, phage culture, 1M CaCl₂, 1M MgCl₂, 1x SM buffer, centrifuge tubes, 1000 µL and 200 µL filter tips, heating block/bath.

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Initial preparation of bacterial culture

- 1 Spread bacterial culture on an agar plate and incubate at required temperature until clear colonies appear.
- 2 Inoculate with a single colony of the bacteria to prepared media, incubate at required temperature, and wait until exponential phase has been reached. Preferably an OD₆₀₀ between 0.2 – 0.5. Then continue to either Full plate plaque assay or spot test plaque assay - depending on the purpose

Full plate plaque assay

- 3 Prepare a 10-fold dilution series of phages in SM-buffer.
- 4 Prepare test tubes with 3-4 mL melted soft agar cooled down to 50°C in a heating block
- 5 Add 300 µL of bacterial culture

- 6 Add 50 µL of 1M CaCl₂ and 50 µL of 1M MgCl₂
- 7 Add 100µL of the 10-fold dilution series made of the phage lysate.
- 8 Do not vortex, but mix by shaking carefully
- 9 Immediately after pour on an agar plate. Let it solidify for 15-20 min or until the lid is clear for condensate water
- 10 Incubate plates at 37°C or other required temperatures.
- 11 Count the plaques and choose the plates with 30-300 plaques to calculate PFU/mL
- 12 $(\text{PFU} / 0.1 \text{ mL}) * (1/\text{dilution}) = \text{PFU/mL}$.

Spot test plaque assay

- 13 Prepare a 10-fold dilution series of phages in SM-buffer.
- 14 Clearly mark on each plate the area for each dilution.
- 15 Prepare test tubes with 3-4 mL melted soft agar cooled down to 50°C in a heating block

- 16 Add 300 µL of bacterial culture
- 17 Add 50 µL of 1M CaCl₂ and 50 µL of 1M MgCl₂
- 18 Immediately after pour on an agar plate. Let it solidify for 15-20 min or until the lid is clear for condensate water
- 19 Deposit 10 µL of phage suspension on top of the marked area. The lid can be slightly opened to speed up the drying.
- 20 Dry plates until the phage solution have evaporated - may take 30-90 min.
- 21 Incubate plates at 37°C or other required temperatures.
- 22 Count the plaques lysis and choose the plates with 10-50 plaques to calculate PFU/mL
- 23 $(\text{PFU} / 0.01 \text{ mL}) * (1/\text{dilution}) = \text{PFU/mL}$