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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

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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 (PFU / 0.1 mL) * (1/dilution) = 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

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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 (PFU / 0.01 mL) * (1/dilution) = PFU/mL

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