Efficiency of Bacteriophage Plaquing (EOP) Assay

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Abstract

Efficiency of plaquing (EOP) assay for *C. jejuni* phages

Jessica Sacher (with help from Stephanie Guerra)

1. For each strain, streak *jejuni* cells from frozen onto NZCYM 1.5% agar plates, grow 2 days in microaerobic incubator.
2. Restreak cells onto a fresh NZCYM plate and grow 1 day.
3. Harvest cells by flooding plate with 5 mL NZCYM broth, mixing with a spreader and collecting resultant suspension.
4. Mix well by inversion and set OD_{600} for each strain to 0.35 (or to 0.0035 – this has also worked well) in 5 mL NZCYM broth in an empty Petri dish (regular size).
5. Incubate at 37°C for 4 h at 100 rpm (place Petri dishes in a large glass beaker inside of an autoclave bag and fill with trigas mixture – beaker should also contain ~50 mL water in a flask which is taped to the side of the beaker, which helps to keep atmosphere moist).
6. During 4-h incubation:
    1. Melt NZCYM 0.6% agar (microwave) and keep at 55°C until ready to harvest cells (let sit at least ~30 mins at 55C prior to use in order to ensure it is not too hot).
    2. Serially dilute phages (10-fold dilutions, usually down to 10^{-5} or 10^{-6}) and store on ice until needed.
    3. Label (including spaces to spot dilutions: 0, -1, -2, -3, -4, -5) and pre-dry (open inside biosafety cabinet for ~30 min) NZCYM plates (one per phage/strain combination).
7. After the 4-h incubation, mix cultures by swirling and measure OD_{600}. Set OD of each strain to 0.3 and add 250 µL of this suspension to a 15-mL Falcon tube (0.5 OD x 200 µL has also worked well).
8. Add 5 mL molten agar to each tube, mix thoroughly but gently (avoid bubbles) by inversion. Immediately pour the entire contents of each tube onto an NZCYM plate.
9. Let solidify ~10-20 mins.
10. Spot dilutions of phages (10 µL x 2 spots per dilution) as well as undiluted phage stock and let plates dry with lids off until spots are absorbed (~15-20 min).
11. Incubate plates upside down in microaerobic incubator for 18-24 h. Check plates for plaques and image using an iPhone (or appropriate substitute). Count isolated plaques and record.
12. To convert plaque numbers to PFU/mL, multiply the number of isolated plaques by the inverse of the dilution factor (ie. If 5 plaques are recorded on the -4 dilution spot, multiply 5 by 10^4, then multiply this number by 100 to account for pipetting only 10 µL – this would give 5 x 10^6 PFU/mL). Shortcut: number of plaques (5, in this example) x $10^{(\text{dilution number plus 2})}$ = 5 x $10^{(4+2)}$ = 5.0 x 10^6 PFU/mL.
13. Graph PFU/mL vs. *jejuni* strain to compare plaquing efficiency of a phage on multiple strains. Alternatively, convert each PFU/mL value to a percentage of a reference (e.g. wild type) strain.

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