One-step growth experiments (cyanophages)

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Abstract

Described is the procedure used to perform a onestep growth curve for cyanophage BBC1-P1 via plaque assay on *Synechococcus* sp. BBC1 (Suttle and Chan 1993).

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Guidelines

The procedure is similar to that for bacteriophages with one major difference. Where bacteriophage growth is measurable in the order of minutes, cyanophage growth curves are measured in terms of hours. The burst sizes are similar, being in the tens to hundreds.

As for bacteriophages, infection should be done at a MOI between 0.1 and 0.01. In this instance, the total infective center = the phage input because the proportion of multiplyinfected host cells is small. At higher MOI, the probability of cells infected by more than one virus would increase and the total infective centers (TIC = total number of infected cells + free viruses) become less than the phage input.

The dilution factor in going from the adsorption tube to FGT is chosen so that a reasonable number of plaques (ca. 50- 200) will form on the plates during the latent period. The dilution factor in going from the FGT (first growth tube) to SGT (second growth tube) is chosen on the basis of the expected increase in plaques at the end of the rise period so that later platings of SGT samples will also yield countable numbers. As mentioned in the bacteriophage section, it is recommended to perform a preliminary one-step experiment to estimate the possible length of the latent period and burst size. Then repeat the experiment with more frequent sampling with adjustments to the dilution factors to gain more precision. Sampling times may need to be adjusted for each phage-□□host system.

In summary:

1. Determine total phage input ($Phage_{input}$) and total cyanobacteria input ($Cyano_{input}$).

2. At T = 0 determine the titer of unadsorbed phages (Free $Phage_{T=0}$) and total infective centers ($TIC_{T=0}$ = initial total infected cells).

3. During the latent period and rise, determine the titer of total infective centers (TIC = total infected cells + any phages released).

4. At the end determine the titer of total progeny (TIC_{final}).
Calculations.

Adsorbed phages = $Phage_{input}$ - Free $Phage_{T=0}$
Percent adsorption = Adsorbed phages/ $Phage_{input}$ x 100
Average MOI = Adsorbed phages/$Cyano_{input}$
Burst size = ($TIC_{final}/TIC_{T=0}$)

Protocol

Step 1.
Set up triplicate adsorption tubes (AT) in 1.5 mL microtubes.

Step 2.
Add phages of a known titer to the cyanobacteria host in exponential growth (cell concentration determined by microscopy; $Cyano_{input}$) at an MOI of approximately 0.02.

NOTES
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(e.g., 0.9 mL hosts [1×10^7 cells mL^{-1}] + 0.1mL phages [2×10^6 PFUs mL^{-1}] = MOI of ~0.02).

Step 3.
Allow the phages to adsorb to the hosts for 60 min. at room temperature and an illumination of 25µmol quanta $m^{-2}s^{-1}$.

DURATION
01:00:00

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Flick tubes a couple of times at 30 min.

Step 4.
Set up control tubes (CT) as above, except:
a. positive controls: replace host cells with media (this gives input phage numbers).
b. negative controls: omit virus

Step 5.
After 60 min, remove unadsorbed phages (T = 0)

DURATION
01:00:00

PROTOCOL

. Remove unadsorbed phages

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Step 5.1.
Centrifuge briefly to pellet host cells (e.g., 5 min, 16,000g, 4°C).

DURATION
00:05:00

Step 5.2.
Remove supernatant, resuspend cells in fresh media.

Step 5.3.
Repeat washing step.

Step 5.4.
Assay washes to determine the number of unadsorbed phages (Free Phage$_{T=0}$) for calculation of efficiency of adsorption.

Step 6.
Prepare nine 15-mL centrifuge tubes containing 10 mL algal growth media

NOTES

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(6 labeled “FGT” [first growth tube] and 3 labeled “SGT” [secondary growth tube]).

Step 7.
Add 100 µL from AT to FGT (10^{-2} dilution).

Step 8.
Mix and remove 0.1 mL sample for T = 0.

Step 9.
Perform plaque assay immediately to determine total infective centers (TIC$_{T=0}$).

Step 10.
Add 100 µL from FGT to SGT (10^{-4} dilution).

Step 11.
Incubate FGT and SGT tubes at room temperature and ca. 25 µmol quanta $m^{-2}s^{-1}$

Step 12.
Remove 100 µL samples every 3 h from FGT (at 3, 6, 9, 12, 16 h) and SGT (from 12 h onwards) for ca. 30 h.

Step 13.
Determine the TIC via plaque assay.

Step 14.
Add 100 µL from positive control tube (CT) to FGT-control (10^{-2} dilution).

Step 15.
Determine the total phage input (Phage$_{input}$).