One-step growth curves for Pseudoalteromonas phages

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Abstract

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Guidelines

Before performing a one-step experiment, you must have documented three consecutive days of consistent growth curves for the host.

Host growth curve:

1.) Inoculate a new culture 1:100; i.e., transfer 80 µl from yesterday's culture to a new tube containing 8 ml of Zobell media. *Pseudoalteromonas* should grow at 21 °C, shaking at 150 rpm.

2.) Immediately after the transfer, take a 'time 0' reading.
a.) Pipet 200 µl of Zobell media into wells A1 and A2 of a white microtiter plate. This is your 'blank'.
b.) Pipet 200 µl of sample (the new culture you just inoculated) into wells B1 and B2 of the same plate.
c.) Ensure that there are no bubbles in the wells, as they will affect your readings. Pipet away any bubbles.
d.) Read the plate on the plate reader.

3.) Continue taking readings in this way periodically. You can start with longer intervals (30—60 minutes) until you start to see growth, then shorter intervals (15—30 minutes) until the growth starts to level off. This should take anywhere from 5 to 10 hours. If it's taking a while, you can go back to reading at longer intervals.
a.) Graph the results as you go!

4.) Take one final reading the next morning.

5.) Inoculate a new culture 1:100 and repeat until you have 3 consecutive days of consistent growth.

You must also determine the titer of your phage lysate before performing the experiment.

1.) Do a plaque assay to determine the PFU/ml of the lysate you plan to use.
2.) Calculate the volume needed for 10^7 If this is less than 1 µl, you will need to dilute your lysate first.

One-step growth experiment (note that this is performed in triplicate; instructions are for MOI=0.1):

1.) First, determine when during the growth of your host you should start the one-step experiment. Compare your 3 growth curves and see when the host is in mid-exponential (log linear) phase. This is the right time to start an infection.

2.) Determine the concentration of your culture at the time you want to start the infection. Use a correlation of readings from the plate reader and cell counts (CFU, DAPI, or FCM counts) to estimate this.

3.) Calculate the volume of host culture needed for 10^8

4.) Pipet this amount into three 1.5 ml tubes, labeled A, B, and C (these are your triplicate infections).

5.) Add 10^7 phages to each tube and start your timer for 15 minutes to allow the phages to adsorb to the host cells.

6.) After 15 minutes, dilute each infection 1:100 in Zobell media in a 250 ml flask (also labeled A, B, and C). These flasks should be in the shaker for the duration of the experiment.

7.) Take a sample immediately after dilution – this is 'time 0'.
a. 0.2 µm filter 1 ml from each flask into a 1.5 ml tube. Use a 25 mm or smaller syringe filter.
i. If your diluted infection is less than 40 ml, you will need to take smaller samples. Never use more 25% of the volume for the experiment (note that you will be taking 10 samples from each flask). You can use as little as 500 µl if necessary.

8.) Continue sampling in this way every 20 minutes for 3 hours.
a. This is a total of 10 time points: T0 – T9.

9.) Store the filtered samples at 4 °

Evaluating samples from the one-step experiment:

1.) Try to analyze the samples as soon after the one-step experiment as possible so they don't degrade.

2.) Calculate the expected concentration of phages at time 0. This will depend on the total volume of the initial infection (i.e., the volume of 10^8 cells plus 10^7 phages). So the concentration at T0 should be 10^7/volume of infection, divided by 100 (for the 1:100 dilution). Convert this to phages per ml.

3.) Once you know how many phages to expect (usually near 10^5), you know what dilutions of your early samples to plate to get good counts. For example, if the T0 expected concentration is 10^5, there should be 100 plaques if you plate 100 µl of a 10^{-2}
a. Keep in mind that some of the phages will have adsorbed to cells and will not show up as plaques, so the actual concentration you calculate at T0 will be less than the expected concentration. This difference gives you the number of phages that infected a cell during the 15 minute adsorption period.

4.) All samples should be plated and counted in triplicate to account for plating errors.

5.) Start by plating one replicate of all 30 samples. Plating all samples on one day reduces errors due to the phages degrading over time. Plate several dilutions of each sample to ensure that you will be able to get a good count.

a. A general guideline is that the phage concentration will increase by 10—100x during the burst, which should be at about the mid-point of the experiment. However, the burst can happen earlier or later and the exact size of it will vary, so give yourself some leeway in the dilutions you plate.

6.) The next day, count the plaques on all plates that have a countable number of them. Decide with dilution gives the best count at each time point.

a. Depending on the size of the plaques, a good count will be somewhere between 10 and a few hundred.

7.) Plate two more replicates of each time point. Use the dilution you decided was best.

8.) The next day, count plaques on the new plates, and also check the first replicate to see if any new plaques have appeared. Add these to your original count.

9.) The next day, check replicates two and three for new plaques.

10.) When reporting results of the one-step, first average the three plating replicates for each time point. Then average the three biological replicates (A, B, and C) and calculate standard deviations for each time point. Graph the results.

11.) Calculate burst size

a. Take the average of the time points on the plateau before the burst (A).

b. Take the average of the time points on the plateau after the burst (B).

c. Subtract A from B. This is the total burst (C).

d. Divide C be the number of infecting phage (expected phages at T0 minus actual). This is the burst size.

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