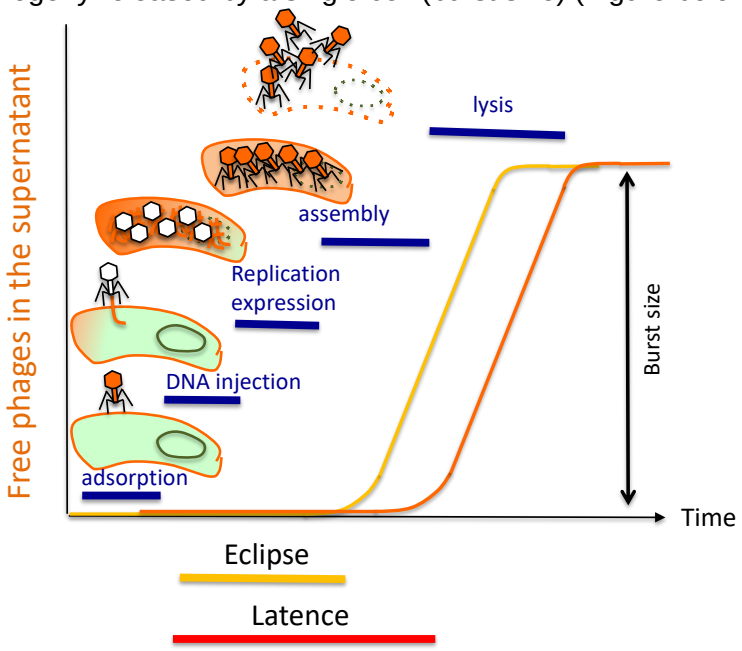
INFECTION DYNAMICS AND EVALUATION OF PROGENY SIZE

Introduction

Phage infection comprises an extracellular stage (adsorption to a specific receptor) and an intracellular stage (injection of DNA into the cytoplasm, hijacking of the cellular machinery for viral gene expression and DNA replication, encapsulation of viral DNA, head-tail assembly, lysis of the cell for release of progeny). The protocol for determining adsorption dynamics is presented in a separate data sheet. Here we use a simplified method to assess the latency time and size of progeny released by a single cell (burst size) (Figure below).

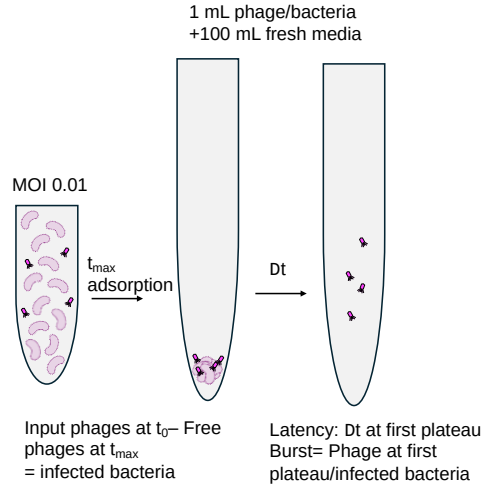


The phage is placed in the presence of bacteria at a low MOI (0.01) to avoid superinfection. The control input (in PFU/mL) corresponds to the same quantity of phage in medium without bacteria. The time required for maximum adsorption (previously determined) is incubated, and the presence of free phage is quantified, i.e. Free phage control (PFU/mL). Input control - free phage control = concentration of adsorbed phage = concentration of infected bacteria (CFU/mL).

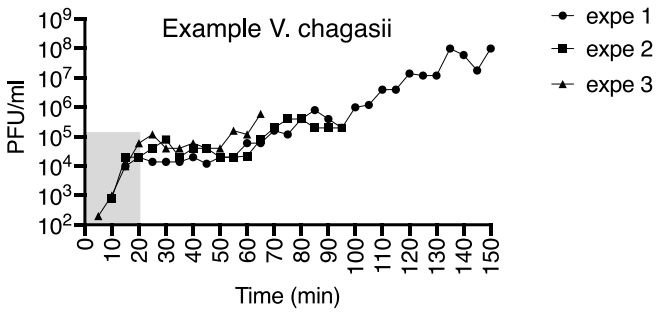
Bacteria are centrifuged to remove excess un-adsorbed phage, and cells are resuspended in a large volume of fresh medium (100X), this time incubated with gentle agitation, and an aliquot (1 mL) is taken at regular time intervals. Each aliquot is centrifuged, the supernatant recovered and either filtered or supplemented with 10% chloroform. Free phages are then titrated.

The number of particles released into the supernatant increases until an initial plateau phase is reached (= lag time, 15 minutes in the figure below). The number of PFU/mL obtained at the plateau divided by the number of infected cells/mL = burst size.

Method



Results



Burst= Phage plateau/infected bacteria

Materials

- Host bacteria
- Phage of interest
- Marine Broth (MB) filtered over 0.2mM to eliminate aggregates
- Erlenmeyers (phage control alone; bacteria plus phages at theoretical MOI 0.01)
- Eppendorf tubes 1.5mL labelled
- Medium petri dish with Marine agar (MA) to titrate the host bacteria
- Big petri dish wtih MA to titrate phages
- Top agar in MB (0.2% for the schizo phages; 0.4% for all the other phages)
- Chloroform

Method

Day -1

- Launch a bacterial culture in 5mL of Marine Broth (MB), ON, 20°C soft agitation (100 rpm).

Day 0

- Dilute the initial culture to 1/100 in 10 mL of MB in an Erlenmeyer.
- When the bacterial suspension has reached a DO 0.3, take 100 ul of the culture, and do limit dilutions (10^{-1} to 10^{-8}) and place a 5ul drop of each dilution on MA agar => this will give you the confirmation of CFU/mL.
- Put 5 mL of the bacterial suspension at DO 0,3 in a small Erlenmeyer or glass tube.
- For the **input control**, put 5 mL of MB media in the same type of container.
- To the bacterial suspension add X µL of phage to have an MOI 0.01.
- Put the same volume of the phages in the **input control**. Mix and let it rest at 20°C until the end of the experiment.
- At t=max adsorption take 1 mL of the bacterial suspension + phage and centrifuge at 6000 rpm for 5 min.
- Obtain the supernatant=> this is the **control of the free phages** (from the supernatant after adsorption), add 100µL of chloroform, vortex and save at 4°C.
- Resuspend the pellet in 1mL of fresh MB and in an Erlenmeyer add fresh MB as needed to make 100ml, incubate in soft agitation (100 rpm) at 20°C.
- Every 10 min, take 1 ml of culture, centrifuge, obtain the supernatant in an Eppendorf tube, add 100uL of chloroform, vortex, and save at 4°C until the titration of the phage.

- At the end of the experiment, take 1 mL of the input control and add 100ul of chloroform.
- Centrifuge the control input tubes, the control free phage and the tubes of the kinetic for 5min at 13 000 rpm.
- Take 100 µl of the supernatant for a limit dilution in 96-well plate (pure to 10^{-7}).
- Place 5ul of each dilution in a bacterial lawn in top agar.

Important tips!

- There is theory and reality. Some phages produce an excellent titre using the “high titer stock” method and large plates, yet they show a small burst (10) at the first plate (e.g. phage P115 from V. chagasii). In fact, it seems that phage growth takes place in several infection plateaus by successive progenies on uninfected cells, making it possible to obtain two more logs of phages after a few hours.

- For some phages, no plateau is observed, and phage multiplication requires at least 6 hours of infection.

- Here too, the notion of low MOI varies from phage to phage. Some require working at MOI <0.001.

References

Zurabov and Zhilenkov *Virol J (2021) 18:9* Characterization of four virulent Klebsiella pneumoniae bacteriophages, and evaluation of their potential use in complex phage preparation.

Pour la technique Gold mais super lourde (donc pas adapté à un grand nombre de phages) : Martha R.J. Clokie et al. (eds.), Bacteriophages: Methods and Protocols, Volume 3, Methods in Molecular Biology, vol. 1681, https://doi.org/10.1007/978-1-4939-7343-9_3, © Springer Science+Business Media LLC 2018