Adsorption of Myoviruses to Resistant Bacteria

Michal Coret & Rachel Sniffen

Mentor: Sophia Zbrowsky, MSc
Department of Biology, Technion – Israel Institute of Technology

Introduction

Our study is focused on the interactions between cyanobacteria and cyanophages.

Cyanobacteria
Cyanobacteria are marine unicellular organisms. They are among the most abundant photosynthetic organisms on the planet. There are two genera: *synechococcus* and *prochlorococcus*. *Synechococcus* is commonly found in coastal waters, and the *prochlorococcus* is found in the open sea.

Cyanophages
Viruses are tiny parasites that are dependent on host cells in order to reproduce. Viruses that attack cyanobacteria are called cyanophages. The two viruses used in this research were T4-like myoviruses; Syn9 and Syn19.

Viral Replication Cycle

A host cell which allows a virus to complete the replication cycle is termed sensitive to the virus. If, at any point, the cycle is stopped, the host cell is termed resistant to the virus.

Objectives

1. Can a virus adsorb to a resistant strain of bacteria?
2. Can it replicate inside a resistant strain of bacteria?

Methods

Adsortion Assay

A method used to check for adsorption by measuring the concentration of the extracellular phage before and after infection.

Polymerase Chain Reaction

The purpose of PCR is to amplify the DNA using a Thermo cycler. The thermo cycler has a set program consisting of denaturation, annealing and extension of the DNA. Denaturation is when the two DNA strands separate under a higher temperature. Annealing is when the primers attach to the target gene. Extension is when the DNA polymerase builds the new complimentary strands.

Quantitative PCR (qPCR)

This procedure involves the use of a probe bound to the template DNA strand. The probe is composed of two fluorescent molecules attached to the complementary DNA. When the polymerase enzyme passes along the probe, it degrades it. This allows the fluorescent molecules to transfer energy between them. The qPCR LightCycler detects this transfer of energy and is able to measure the amount of DNA replicated.

Results

**Figure 1.** (A) An image of *prochlorococcus* from Chiisolmn Lab. MIT. (B) An image of a cyanophage adsorbed to the cell wall, from Lindell Lab, Technion.

**Figure 2.** Viral replication cycle inside the cell. (1) Adsorption recognition followed by attachment to a cell surface receptor by a virus; (2) Entry: injection of viral DNA into the host cell; (3) DNA replication: infected cell makes copies of the viral DNA; (4) Transcription and translation: production of viral proteins; (5) Phage assembly: proteins assemble to produce new viruses; (6) Host cell lysis: host cell bursts to release new viruses.

**Figure 3.** PCR reaction. Source: Bitesize Bio

**Figure 4.** qPCR reaction. Source: Bitesize Bio

**Figure 5.** Adsorption assay results. Resistant *Synechococcus* or *Prochlorococcus* strains were infected with *myovirus* Syn9 or Syn19. Decline in extracellular phage concentration indicates adsorption to the bacterial cells. (A) Positive control: adsorption to a sensitive strain. (B) Negative control: no cells, therefore no adsorption. (C) Syn9 does not adsorb to WH5701. (D) – (F) Syn19 adsorbs to resistant *Synechococcus* strains, (G) – (I) Syn9 adsorbs to resistant bacterial strains.

**Figure 6.** PCR amplification of g20. Lane 1: Lambda hindlader; lane 2: 100bp ladder; lane 3: g20 mix with *Syn9* DNA; lane 4: g20 mix with *Syn9* DNA; lane 5: g20 mix with TIM40 DNA; lane 6: g20 mix with TIM40 DNA; lane 7: g20 mix with no template control; lane 8: g20 mix with no template control; lane 9: g20 miix with PSSP7 DNA; lane 10: g20 mix with PSSP7.

**Figure 7.** qPCR amplification of g20. WH5701 was infected with Syn9. Amplification of g20 shows replication of viral DNA inside the resistant cell.

Discussion

- As seen in Figures D-I, the negative trends indicate no adsorption of the viruses to the bacterial cells. The resistance mechanism of these strains must be at a later point in the cell cycle. It is still unknown as to exactly where the viral replication cycle stops.
- Figure C shows no distinct negative trend, so it can be interpreted that Syn19 does not adsorb to WH5701. A lack of adsorption signifies extracellular defense against the virus, since no recognition and binding of the virus to the receptor on the bacterium occurred.
- For bacteria, the advantage is that they do not undergo lysis.
- Myoviruses are known to have several hosts. This is beneficial for them because they have more potential to reproduce. The trait that is responsible for the viruses’ ability to recognize receptors on sensitive strains is the same trait that allows them to recognize receptors on resistant strains.

Conclusions

Viruses can adsorb to and replicate in resistant strains of bacteria. Further research can be done to identify the specific defense mechanisms that prevent the completion of the viral cycle.

Acknowledgments

Thank you to Lindell Lab for providing us with facilities and to the SciTech organizers for making this all possible. Thank you to our mentor Sophia Zbrowsky.

References: