Bacteria vs Phages: The Never-Ending War

Resistance to Phages in Marine Bacteria

Shira Shanskhalil and Dvora Sehtman; Mentor: Sophia Zborowsky, MSc.

This project was conducted at Prof. Debbie Lindell's lab, Faculty of Biology, Technion

Introduction:

Cyanobacteria are the most abundant photosynthetic creatures on Earth, responsible for the production of approximately 13% of the oxygen on Earth[1]. The cyanobacteria use a wide range of intercellular and extracellular defense systems, which stop the cyanophage infection at different stages[2] (Img.1).

There are two different types of phages: **generalists and specialists**. Generalist phages infect various different hosts, while specialists only attack one specific type of bacteria[3]. Previous research has demonstrated that cyanobacteria normally stop specialist phages before they even attach to the cell: extracellularly. However, some specialist phages can attach to their bacterial host and are stopped intracellularly.

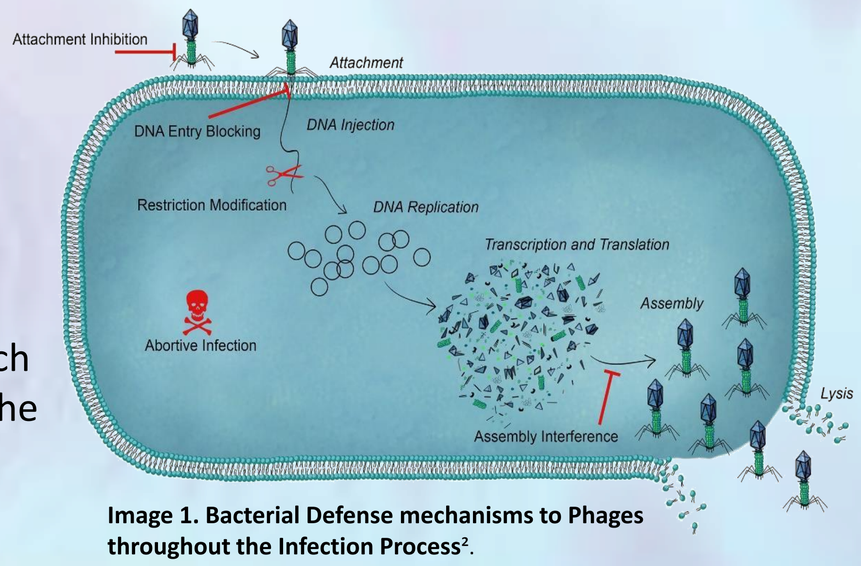


Image 1. Bacterial Defense mechanisms to Phages throughout the Infection Process[2].

Objectives:

Find in which stage the infection of 2 different phages is halted in the resistant bacteria.

Estimate which type of defense mechanism is responsible for the resistance.

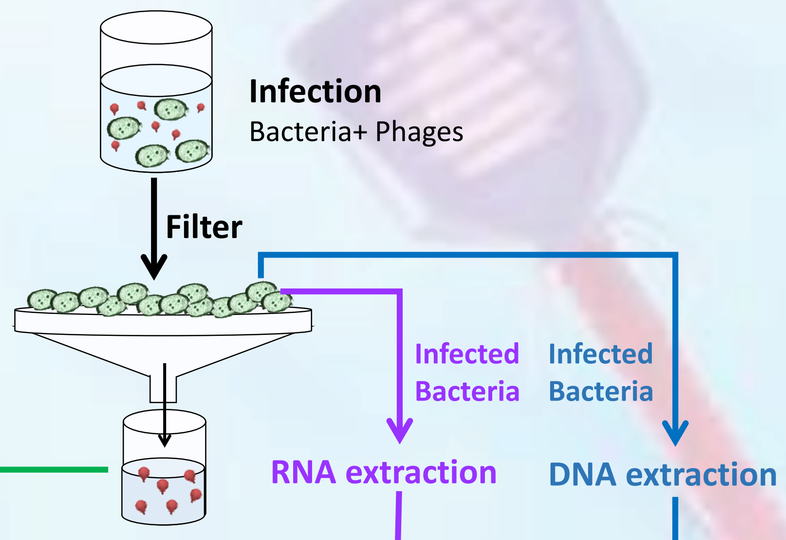
References:

(1) Flombaum, P., Gallegos, J.L., Gordillo, R.A., Rincon, J., Zabala, L.L., Jiao, N. *et al.* (2013). Present and future global distributions of the marine cyanobacteria *Prochlorococcus* and *Synechococcus*. *Proc Natl Acad Sci U S A*, 110: 9824-9829.

(2) Stern, A. & Sorek, R. (2011). The phage-host arms race: shaping the evolution of microbes. *Bioessays* 33:43–54.

(3) Sullivan, M.B., Waterbury, J.B. & Chisholm, S.W. (2003). Cyanophages infecting the oceanic cyanobacterium *Prochlorococcus*. *Nature*, 424: 1047-1051.

Methods:



Adsorption Assay

Quantification of free phages using Plaque Assay.

Plaque assay

Plating bacteria with free phages.

The bacteria grow to form a lawn on the plate. Phages cause clearings in the lawn, called plaques. Plaques are counted to quantify the phages.

Reverse Transcription

Using a Reverse Transcriptase enzyme to transform RNA into cDNA.

Quantification of phage genes using Quantitative Real Time PCR.

- Prepare a solution containing specific primers, DNA polymerase and SYBR green (I).
- The DNA polymerase amplifies the existing DNA targeted by the chosen primers .
- The qPCR is able to quantify the DNA, based on the fluorescent signal emitted by the SYBR green attached to the newly formed double stranded DNA.

Discussion:

MIT9313:
The presence of early gene transcription as well as the minimal DNA replication indicates that MIT9313's defense mechanism inhibited DNA replication, perhaps in a system resembling DNA Restriction Modification systems.

MIT9215:
RNA transcription and DNA replication were observed. Therefore, the resistance mechanism must be in the later stages of infection, such as transcription inhibition of late genes, translation interference, or assembly interference.

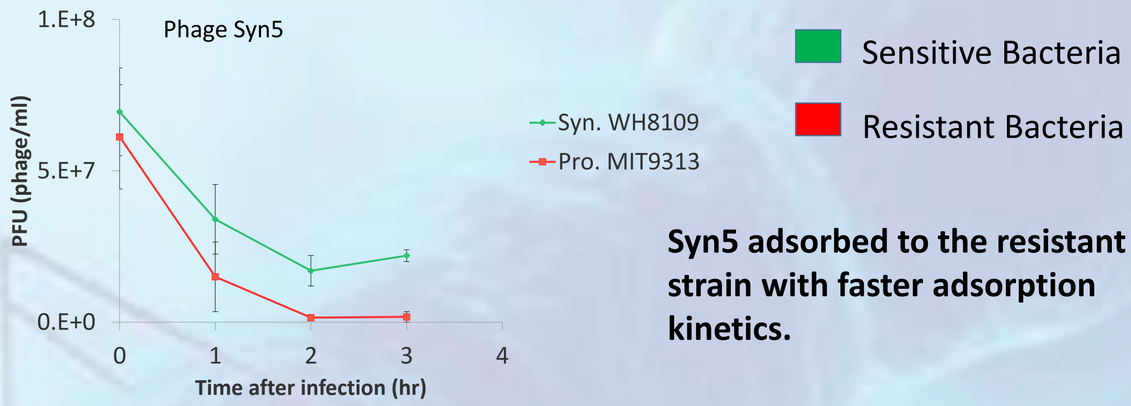
MIT9312:
The minimal amount of early gene transcription as well as the lack of DNA replication might indicate one of the following defense mechanisms: incomplete inhibition of DNA entry, DNA restriction, inhibition of early gene transcription, or blocking of DNA replication.

Significance:

- There is no developed genetic engineering method for *Prochlorococcus.*
- It is possible to genetically engineer phages inside a *Synechococcus* host.
- We can potentially use the genetically modified phages to express genes in resistant *Prochlorococcus* strains.
- Genetically engineering of *Prochlorococcus* can, for example, improve its energy generation efficiency.
- **These results can be used to develop new methods to genetically engineer *Prochlorococcus* strains.**

Results:

Adsorption to Resistant Strains



Syn5 adsorbed to the resistant strain with faster adsorption kinetics.

Fig.1. Bacterial cells were infected with phages and the phages were filtered out. The phages were put on a bacterial lawn and enumerated. A decline in the number of phages indicates an increase in phage adsorption to the bacteria.

Production of RNA in Resistant Strains

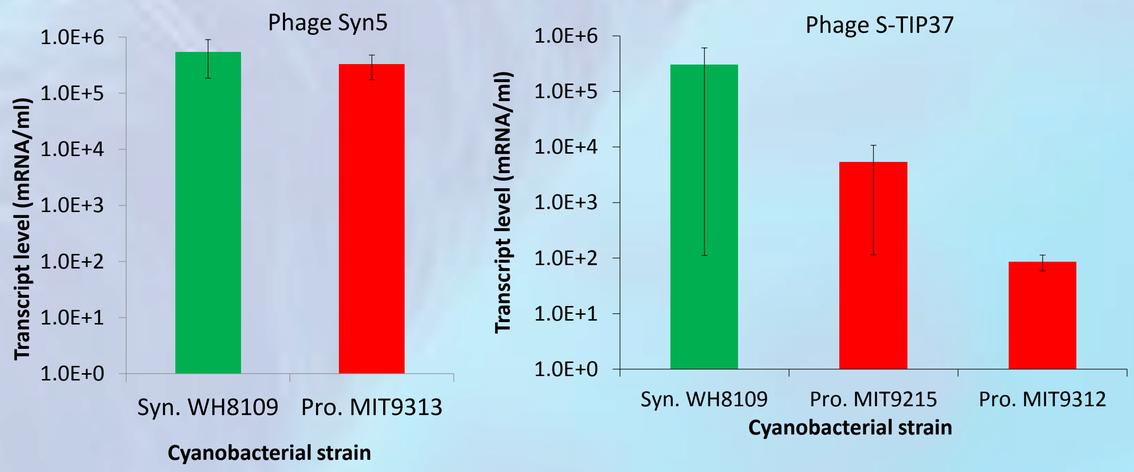


Fig.2. Bacterial cells were infected with phages and the cells` RNA was extracted. If phage RNA was found, the phage DNA entered the cell and the infection was stopped intracellularly. If not, the bacterial extracellular defense mechanisms stopped the phage from entering into the bacteria's cytoplasm.

Syn5 and S-TIP37 phages entered the cell and the bacteria stopped the infection intracellularly.

DNA Replication in Resistant Strains

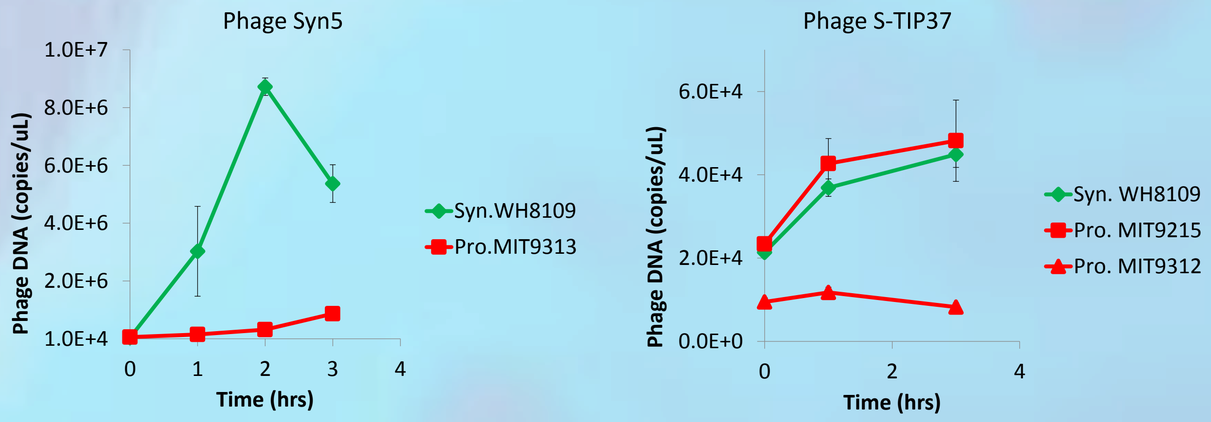


Fig.3. Bacterial cells were infected and their DNA was extracted. If the amount of phage DNA increased with time, the phage replicated its DNA. If not, the bacterial cell stopped the infection earlier.

Syn5 replicated its DNA in the resistant strain and S-TIP37 replicated its DNA in 1 of the 2 resistant strains.

Acknowledgements:

We would like to thank our mentor Sophia Zborowsky, MSc. And Prof. Debbie Lindell for hosting and guiding us through our research in her laboratory.

We would also like to thank the foundations and donors for their generous support of the SciTech program, as well as the SciTech Team for bringing the program to life.