THE INFECTION OF FRESHWATER CYANOBACTERIA BY THEIR PHAGES

A Senior Scholars Thesis

by

LAURALEE M. SHANKS

Submitted to the Office of Undergraduate Research
Texas A&M University
In partial fulfillment of the requirements for the designation as

UNDERGRADUATE RESEARCH SCHOLAR

April 2007

Major: Biochemistry and Genetics

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Approved by:

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Associate Dean for Undergraduate Research: Robert C. Webb

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ABSTRACT

The Infection of Freshwater Cyanobacteria by Their Phages (April 2007)

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Phage lysis of cyanobacteria could significantly influence nutrient availability in aquatic systems. We examined the requirements of phage infection in freshwater cyanobacteria and identified several factors that affected infectivity. Light was found to be required for the infection of a wild cyanobacterial isolate (*GH6*) by bacteriophage AS-1. An identical requirement was found for the infection of *Nostoc* by its phage An-29. In both the wild isolate and *Nostoc*, the absence of light during infection reduces the yield of progeny phages to the background over the course of two rounds of infection. In addition, filtering out different portions of the visible light spectrum results in reduced progeny yield. The culture density was also found to strongly influence the ability of AS-1 to infect *S. elongatus* and the wild isolate. Phage progeny production and the timing of progeny release were influenced by culture density.

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Most importantly, I would like to thank Dr. Cheng Kao for giving me the opportunity to conduct research in his lab; without his guidance and support, I would not have been able to accomplish all that I have with my research. I also want to extend my gratitude to the Kao Lab, The Cereal Killers, for all of their patience, assistance, and direction over the past few years.

NOMENCLATURE

mL	Milliliter
OD	Optical Density
PFU	Plaque-forming units
TAMU	Texas A&M University
μL	Microliter

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CHAPTER I

INTRODUCTION

Photosynthetic prokaryotes are extremely abundant and widely distributed in aquatic ecosystems on Earth (11). Cyanobacteria, or blue-green algae, are the oldest photosynthetic prokaryotes and account for approximately 8% of the entire bacterial population in waters around the world (10). These highly diverse gram-negative organisms exhibit the ability to conduct oxygenic photosynthesis, a primary mechanism for utilizing the Earth's solar energy and the cycling of atmospheric carbon and oxygen (3) . In addition to cyanobacteria, bacteriophages are copious inhabitants of marine and freshwater environments. These organisms are suggested to be "the most abundant biological entities on the planet", having significant influences on biogeochemical cycles and genetic diversity in bacterial populations (8).

It is estimated that 90% of all biological carbon in salt water bodies is in the form of cyanobacteria and archae, which together are typically referred to as bacterioplankton (8). Cyanobacteria, such as those of the genera *Synechococcus*, are extremely influential in carbon dioxide fixation and oxygen production of such ecosystems. Although the population of these organisms is large, approximately 10^7 cells per milliliter water, the

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This thesis follows the style of Applied and Environmental Microbiology.

bacteriophage population tends to be an order of magnitude higher (8). This is significant in the fact that bacteriophage infection of such important ecosystem members affects the cycling of organic carbon. Therefore, it is important that we understand the mechanism by which phage infection of cyanobacteria occurs.

Because cyanobacteria are photosynthetic organisms, they require light for metabolism. Extensive research has been conducted in order to identify and understand the method of action of photoreceptors present in these organisms (9), as well as to determining the effects of light intensity on their operation (7). Scientists also seek to understand the role of light in cyanophage infection (1, 2). It is known that in addition to being needed for growth and metabolic functions, the presence of light is required for the infection of *Synechococcus elongatus* (PCC 7942), a freshwater cyanobacterium, by its contractile bacteriophage AS-1 (6). In our studies, we investigate whether or not this requirement holds true for other freshwater cyanobacteria.

Few research efforts have been concerned with the effects that culture density could have on cyanobacterial growth, and no previous studies were found that examined the possible effects on phage infection. However, we report the observation of a correlation between cell density and AS-1 infection in *S. elongatus*. Culture density appears to be inversely related to phage progeny production and influences the timing of phage lysis.

CHAPTER II

METHODS

Cyanobacteria and bacteriophage strains

Synechococcus elongatus PCC 7942 was a kind gift of Dr. Susan Golden. AS-1, *Nostoc* sp and bacteriophage An-29 were all obtained from the American Type Culture Collection of Cyanobacteria.

Isolation of wild cyanobacterial colonies

Wild cyanobacterial strains were isolated to test along with *S. elongatus*. Water samples were collected from several freshwater sources around Bryan/College Station, including Lake Bryan and Texas A&M University's Research Park. Aliquots of the samples were spread onto BG-11 agar and incubated to allow organisms to grow. Green colonies indicative of a possible cyanobacterial species were streaked onto BG-11 agar, and each culture passed through several rounds of streaking to ensure that a pure culture was obtained.

A cross-streak assay to test sensitivity to AS-1

Isolates that could be infected with bacteriophage AS-1 and/or An-29 were identified using a cross-streak assay. Briefly, isolates were streaked perpendicular to a recently dried patch of a phage lysate on a BG-11 plate. After incubation, clearing in the growth of the cyanobacteria where the phage was dripped indicated successful phage infection.

GH6, which is capable of phage infection by AS-1, is one such isolate found in the TAMU Research Park sample. Infectivity of GH6 by AS-1 was confirmed by checking for plaque formation using the phage titering assay discussed below.

Growth conditions and density measurements

All liquid cultures were grown in an incubator under constant illumination on an orbital shaker. Cultures were prepared with BG-11 medium in flat culture dishes. Before infection, the OD of cultures was measured at 595 nanometers using a UV-visible spectrophotometer; 0.5 mL aliquots of the culture were typically used for measurements, and BG-11 medium was used as a blank.

Phage lysate preparation and infection

A stock solution of phage lysate was prepared using the method in (6). An early log phase culture of *S. elongatus* was infected with AS-1 and kept in the incubator until it completely lysed. The supernatant was then purified twice by ultracentrifugation. The stock solution, which was approximately 10^8 PFU/mL, was stored at 4°C with a drop of chloroform. For infection, we added AS-1 to cyanobacterial cultures of desired density and kept them in the incubator under the previously stated conditions until lysis occurred. Phage titering was conducted using a plaque assay in which 1.5% BG-11 agar was overlaid with a few mL of 0.75% BG-11 agar, 200-500 μL of *S. elongatus*, and 20-200 μL of diluted phage lysate (6).

CHAPTER III

RESULTS

We first wanted to test the hypothesis that light is a universal requirement for phage infection of cyanobacteria. In order to do this, we grew early log phase cultures of GH6 and *Nostoc* sp and infected them with bacteriophage AS-1 and An-29, respectively. Once the phage was added, both cultures were divided into two aliquots, one of which was covered with aluminum foil before being placed in the incubator. After approximately 11 hours, the time normally required for completion of one round of infection, the phage titer was determined for each aliquot. The titer for the aliquot kept in light was compared to that of the culture kept in the dark. The data in Table 1 demonstrates that phage progeny production is negatively affected by the absence of light in these two cyanobacteria.

TABLE 1. Light is required for efficient phage progeny production in *Nostoc sp.* and a wild cyanobacterium isolate (GH6)

Cyanobacteria Strain	***Phage***	***PFU/mL (Light)***	***PFU/mL (Dark)***	***Fold Difference (Light/Dark)***
Nostoc	An-29	5.6e9 ± 1.7e9	3.4e3 ± 529	1,637,255
GH6	AS-1	3.6e9 ± 7.8e8	4.1e4 ± 707	87,654

To investigate the effects of culture density on cyanophage infection, a culture of *S. elongatus* was grown to an optical density (OD) greater than 1.0. Serial dilutions of this culture were made using BG-11 medium to achieve cultures with densities of approximately 0.3, 0.5, and 0.7. The diluted cultures, as well as the original, were infected with AS-1 and the phage titer was determined after one round of infection. Plotting OD vs. PFU/mL (Figure 1), we observe a negative relationship between OD and the number of phage progeny released after one round of infection.

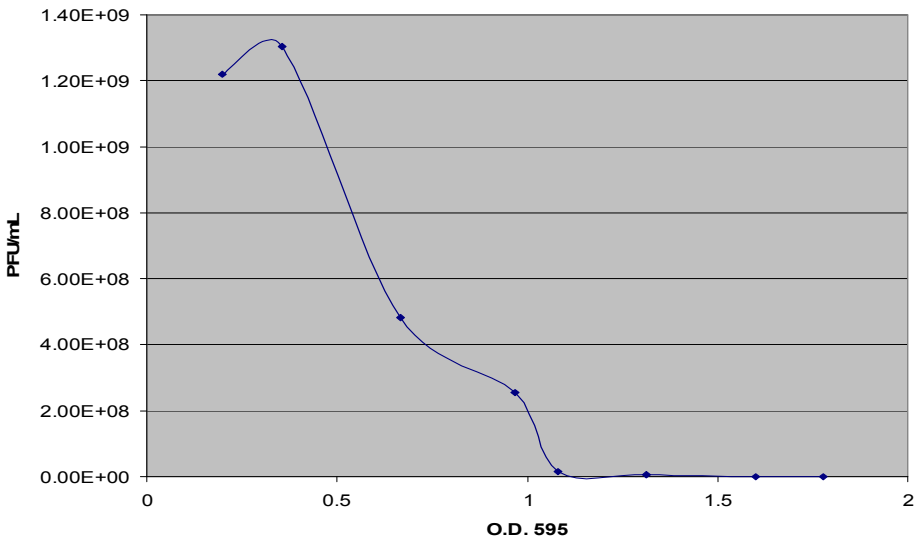

FIG 1. The OD of a culture is inversely related to the amount of progeny phase released after one round of infection

To further examine the relationship between culture density and phage infection observed above, we chose to track the OD of a set of cultures over time. Cultures in the same manner as above; we did not, however, determine the phage titer after 11 hours.

The OD of three cultures was recorded every two hours until lysis was observed, and then phage titers were determined by the plaque formation assay. When OD is plotted versus the time after addition of AS-1 (Figure 2), a positive correlation between OD and the amount of time required for one round of infection was observed.

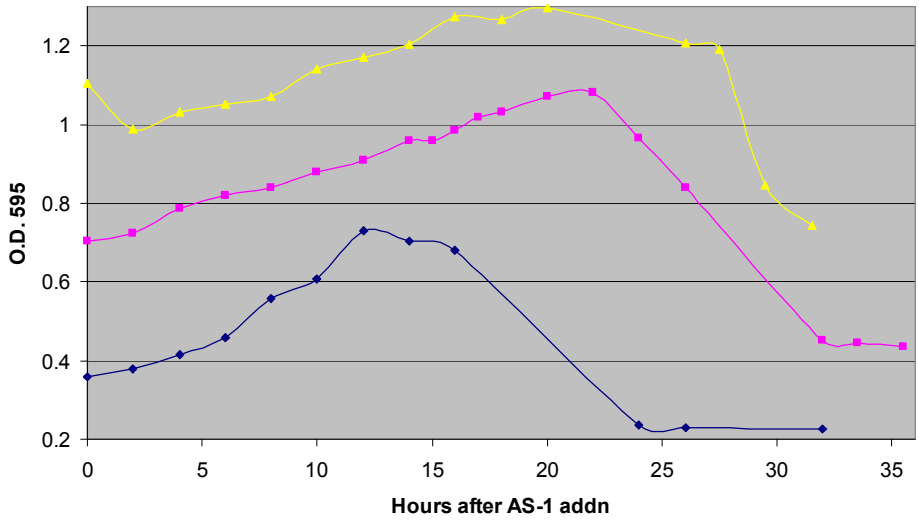

FIG 2. An increase in culture density is accompanied by an increase in the amount of time required for phage lysis in one round of infection

CHAPTER IV

SUMMARY AND CONCLUSIONS

Over the past few decades, several attempts have been made to model the contribution that cyanobacteria make to the Earth's oxygen levels. One such model suggested that one gram of the cyanobacterium *Anacystis nidulans* and its progeny, under ideal growth, space, nutrient and light conditions, would only take 40 days to produce the amount of oxygen presently in the Earth's atmosphere (5). Along with their role in the cycling of organic carbon, cyanobacteria have proved to an extremely important and diverse life form.

We have demonstrated that light and density can influence the ability of bacteriophages to infect several freshwater cyanobacterial species. Light is now known to be required for the infection of two different genera of cyanobacteria and three different isolates, supporting our hypothesis that this is a universal requirement for phage infection in all cyanobacteria. We also observed that filtering out different portions of the visible light spectrum can be detrimental to phage progeny production. In addition to light, we observed several effects that culture density can have on the infection process. The initial experiments showed a negative correlation between OD and phage progeny production, leading us to believe that the cyanobacteria may reach a "phage resistant" state. Further experimentation allowed us to see that culture density simply influences the timing of phage progeny release. Nevertheless, the effects of light and culture density

on phage progeny production are extremely important in aquatic ecosystems. Both factors can serve as mechanisms for controlling the cyanobacterial population and maintaining a nutritional balance in the natural environment.

The mechanism of the phenomenon discussed above remains to be studied. In the future, it will be important to identify the proteins involved in sensing light and to determine the signaling pathway needed to affect phage infection. Furthermore, our sampling of cyanobacteria and phage species should be expanded to examine the generality of light-dependent phage infection. The influence of culture density on phage infection and cell lysis must also be further investigated in order to fully interpret the results we are seeing. For example, is the longer time for lysis due to a change in energy levels in the cell or to a more active mechanism that is suppressing lysis? Lastly, cyanobacteria are known to undergo gene regulation that is regulated by a circadian rhythm (4). It will be intriguing to determine whether phage infection is actively regulated by circadian rhythm. All of these events could vastly influence a process that has significant impact on global nutrient cycling and the health of our planet.

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