The Effects of Ultraviolet Irradiation on the Lytic Capabilities of Bacteriophages.

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Abstract

The bacteriophage is a specialized virus that infects bacteria. Phages are used extensively in research, especially genetic research because they are easily manipulated and present fewer difficulties to work with than other viruses might. They may also serve as models to show how certain experiments or techniques can be used on other viruses. The purpose of this project was to observe how ultraviolet irradiation affects the reproductive capabilities of bacteriophages. The main variables of this project are the ultraviolet light and the concentrations of phage used. Both of these variables caused the reproduction of phages to decrease. The more exposure to ultraviolet light the phage gets the less reproduction there is. The smaller the concentration of phage cells there are the less reproduction there is. The T4 bacteriophage which infects Escherichia coli bacteria was used in this project. The phage was diluted in triptych soy broth to concentrations ranging from 10^7 viral cells per milliliter to 10^{-5} v.c per milliliter. These concentrations are a short hand way of writing out how many viral cells there are per milliliter of tryptic soy broth. The concentrations that were actually used in the project were 10^7, 10^4, 10^1, 10^{-2} and 10^{-5}. One milliliter of the diluted phage was taken and exposed to either 10, 20, 30, or 40 seconds of ultraviolet light. The one milliliter of phage was then mixed with 20µ E.coli and 2ml of triptych soy soft agar and then poured onto a plate of triptych soy hard agar. This allowed for plaques to form showing where the phage had reproduced. The results of these tests show that the phage reproduces less as it is exposed to more ultraviolet light. The phage at a concentration of 10^7 was exposed to 10 seconds of ultraviolet light and produced 33 plaques while the same concentration of phage exposed to 40 seconds of ultraviolet light only produced 7 plaques. There is also a decrease in

phage reproduction as the phage concentrations decrease. The concentration 10^7v.c. per ml had 33plaques after 10 sec of U.V exposure while the concentration 10^4v.c. per ml had 15 plaques after 10 sec of exposure.

Introduction

The research that was carried out in this project involved the use of Escherichia coli bacteria, T4 bacteriophage, and ultraviolet radiation. To fully understand how these things were involved in the project you will need to understand the bacteria, the phage, and the type of radiation being used. The key parts of what you will need to understand are as follows.

1. You will need to understand what Bacteriopahges are and what they do.

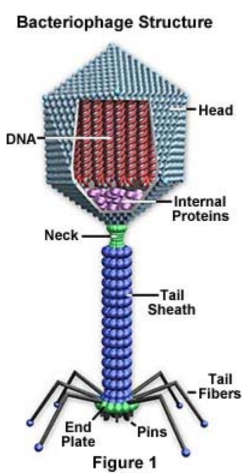
2. The type of reproductive cycle the phage in the experiment will be using. This is very important as the whole experiment is on how the irradiation will affect the Bacteriophages reproductive capabilities.

3. What type of bacteria E. coli are and how they are used in this experiment.

4. What ultraviolet light is and its effect on living organisms.

Bacteriophage or phage, for short, is a relatively small type of viruses that only infects bacteria. The phage part of the name comes from the Greek word phagein, which means to eat. The Bacteriophage was first discovered in 1915 by Frederick Twort but Twort did not pursue the discovery so nothing came of it (Wikipedia Phage). He actually discovered bacteriophages by accident. Twort had been growing viruses for several years when he noticed that some bacteria that had infected his Petri dishes had lysed showing that something had destroyed them. These viruses were not given the name bacteriophage until 1917 when Félix d'Herelle discovered them and started the first research on them (Wikipedia Phage). They are very similar to viruses that infect eukaryotes like plants and animals. They have outer protein shells called capsides which encloses genetic material either DNA or RNA. DNA is in 95% of known phages and is typically double stranded. The average phage will also have 5-650kbp (Kilo base pairs) and will be 24-200nm in length. Attached to the capsid is the tail and tail fibers. These allow the phage to move and attach to a host bacterial cell and inject ether the DNA or RNA. The type of page I will be using in this research is a T4 bacteriophage. It has one of the longest strands of DNA for a phage, 169-170kbp. It belongs to the Myoviridae family of bacteriophages. The T4 also has some unique characteristics. It has a special DNA copying engine being able to copy DNA at record speeds and having only one error out of three hundred copies (Enterobacteria phage T4).

Figure 1



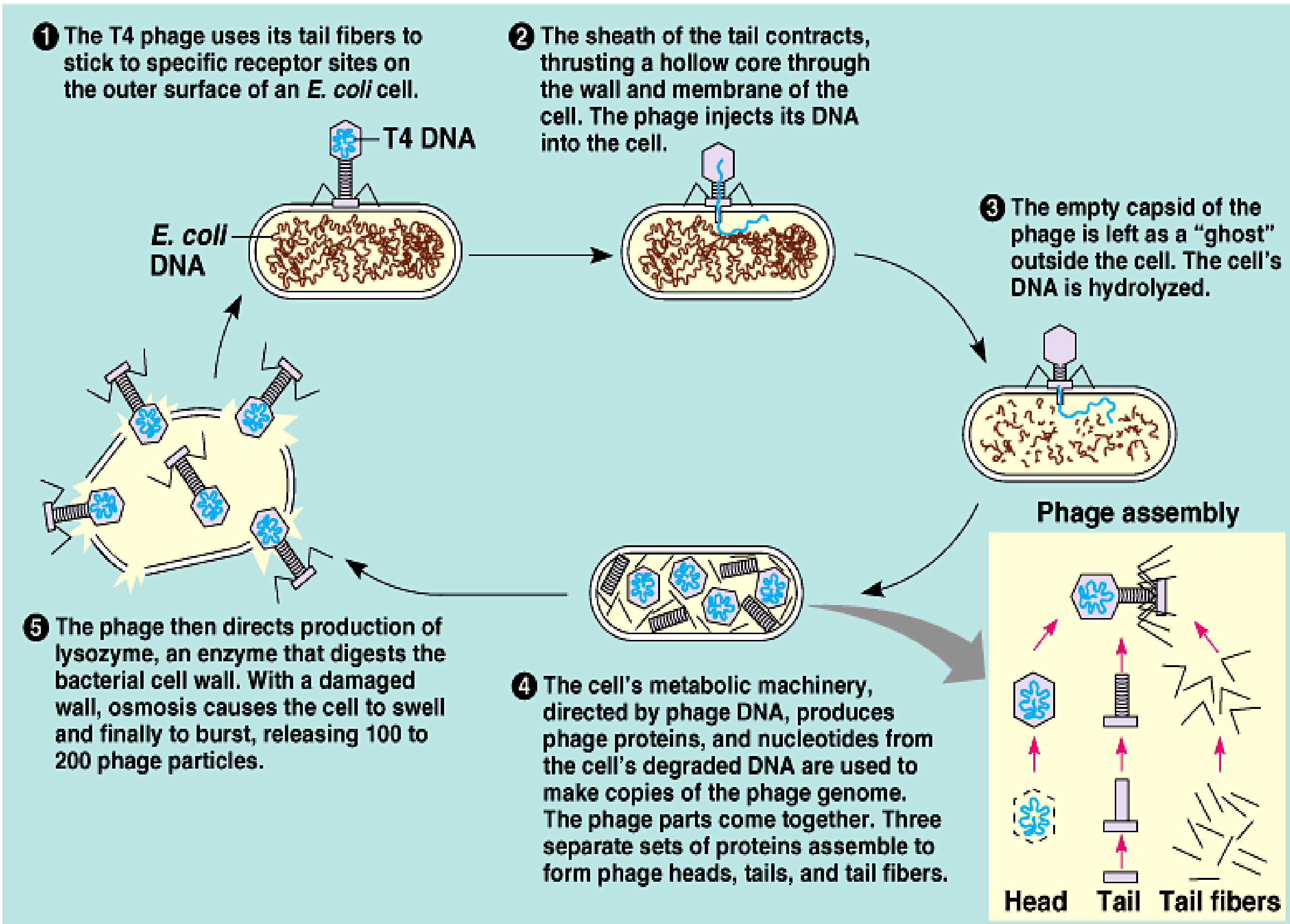
Molecular Expressions

Legend: The structure of a bacteriophage.

Bacteriopahges only infect specific types of bacteria and there is probably a phage for every bacterium on the planet. A phage will infect bacteria and then go into one of two modes of reproduction: the virulent or lytic mode and the lysogenic mode. The T4

Phage used the lytic form of reproduction. The T4 only infects *E. coli*. In the lytic form of reproduction the virus attaches itself to the bacterial cell wall using the tail fibers. The virus then changes shape forcing the hollow tail core into the cell. It then injects the viral DNA into the bacteria. The viral DNA attaches itself to the bacterial DNA and uses the bacteria to make new copies of the virus. The DNA first goes through transcription, which makes RNA. Transcription is the process of synthesizing RNA from a DNA template resulting in the transfer of genetic information from the DNA molecule to RNA. The RNA is just a short copied segment of the DNA. The RNA then goes through translation and makes the necessary proteins for a new virus (Answres.com). It continues this process until there are so many viruses in the cell that it goes through lysis bursting apart releasing the new viruses. After the viruses escape from the bacterial cell they spread out infecting new bacteria and going through the same process.

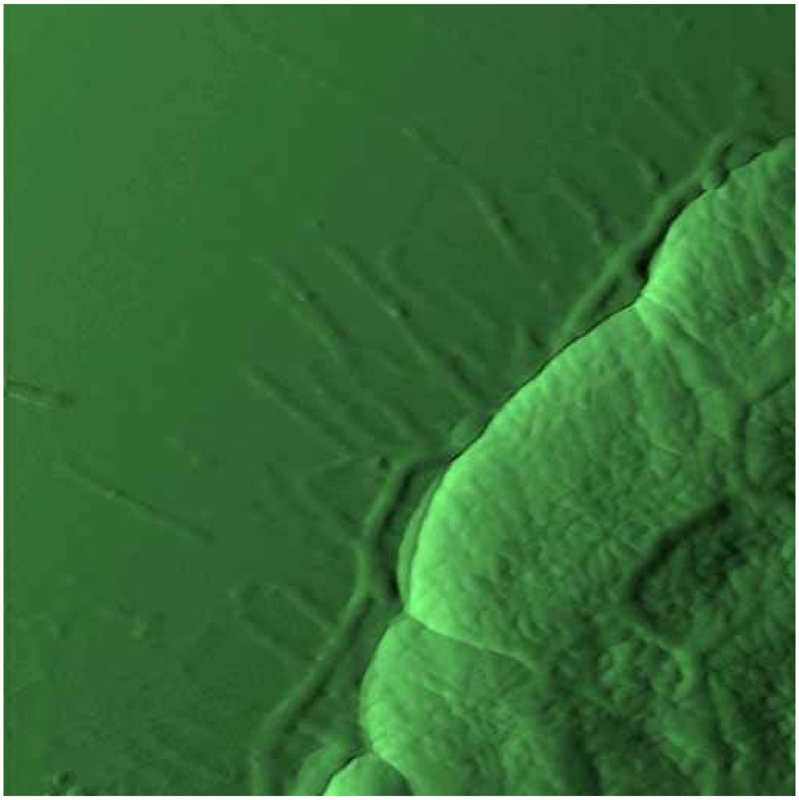
Figure 2



Legend: This picture depicts the lytic process of reproduction for the T4 bacteriophage.

A common type of bacteria is Escherichia coli other wise known as E. coli. It is the most common member of the genus Enterobacteriaceae. It belongs to the family Enterobacteriaceae because the name Enterobacteriaceae comes from the Greek word enterikos which pertains to the intestines where most E. coli are found. It can be found in the intestinal tracts of humans and animals but if it gets into other parts of the body it can cause infections (Answres.com). E. coli can also be found in the mouth, gut, in plain soil, and water. It is rod shaped and moves around with the use of rapidly moving flagella. It is widely preferred in laboratory research especially with genetic engineering research. It is said to be the most researched organism on the planet (Answres.com).

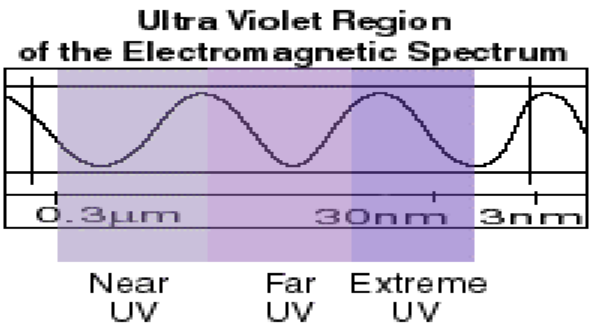
Figure 3



Legend: This picture shows part of the cell wall of an E.coli bacteria cell.

Ultraviolet light cannot be seen by the naked human eye. Its wavelength is shorter than that of ordinary light putting it higher on the electromagnetic spectrum (The Electromagnetic Spectrum). The spectrum for ultraviolet light itself is separated into three regions the near, far, and extreme. The ultraviolet light that will be used is probably more toward the right of the spectrum. Ultraviolet radiation is known to cause covalent bonds to form between adjacent thymine bases in human skin DNA this causes thymidine dimers. The thymidine dimmers cause a distortion in the double DNA helix this leads to stalls in replication and gaps in the DNA.

Figure 4



Legend

This image shows the electromagnetic spectrum for ultraviolet light.

Materials

Chemicals/Consumables	Supplies	Equipment
E.coli T4 Phage at concentration 10^8 Tryptic soy powder Agar powder Distilled water	Test tubes Pipettes Agar plates Test tube caps	Incubator Shaking water bath Autoclave Scale Bunsen burner Box Ultraviolet light

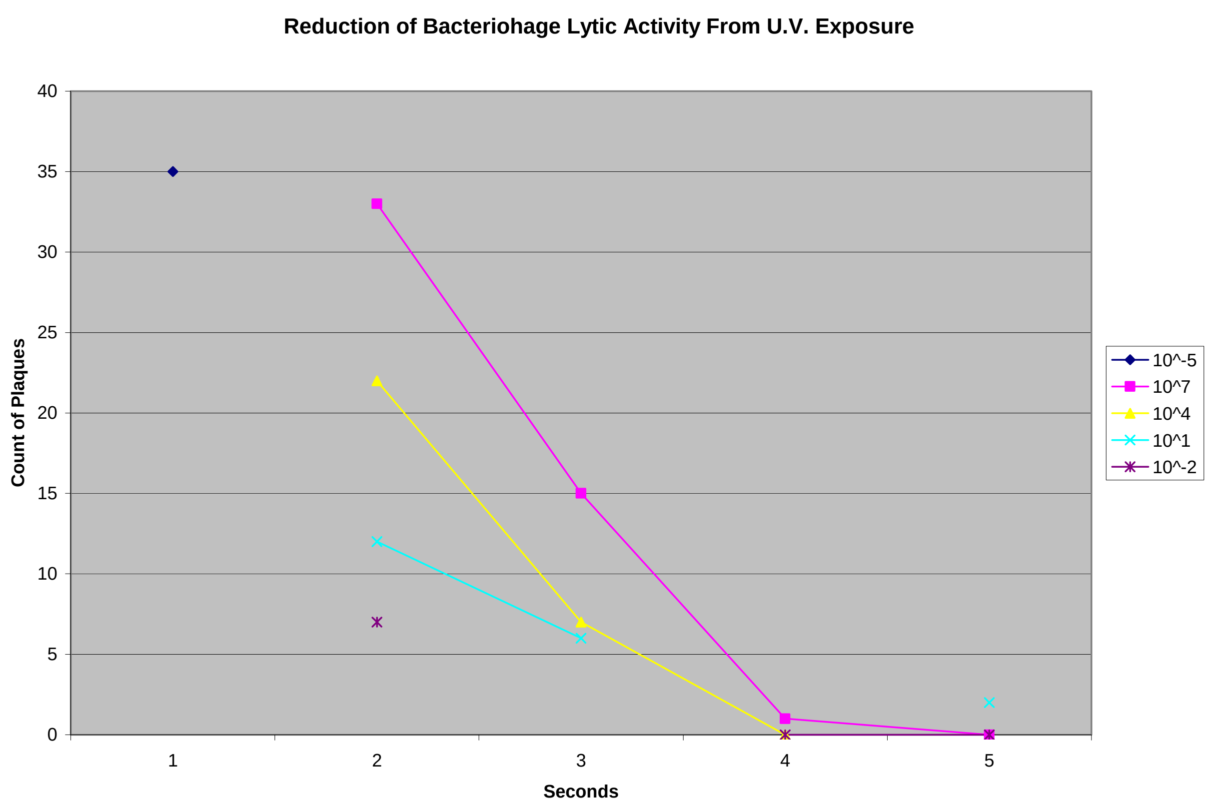
Procedure

Inoculate 10ml of tryptic soy broth with E. coli bacteria using sterile transfer procedure. Grow in a 37 C shaking water bath over night. Dilute bacteriophages T4r to concentration of 10^7, 10^4, 10^1 10^{-2}, 10^{-5} viral cells per milliliter in tryptic soy broth. This is done by transferring one milliliter of the original concentration 10^8 into a test tube with nine milliliters of tryptic soy broth creating the concentration 10^7. You do the same thing with the new concentration and you keep doing it until you reach the desired concentration. Transfer 3ml of tryptic soy soft agar into 17 test tubs. With micro pipet take 20µ of E.coli and put it into 3ml of melted soft agar in a test tube. Then take 1ml of the diluted phage sample 10 and put it in the test tube with the E. coli and soft agar. Pour the soft agar, E.coli and phage on to a plate of hard tryptic soy agar. Let the soft agar congeal on top of hard agar and then place in incubator at 37 C over night. Titer the plaques on the next day. Repeat the above four more times but expose the virus (phage) to 10, 20, 30, 40 seconds of ultra violet light before it is placed with the E.coli in the soft agar. Repeat all of the above but with different concentrations.

Results

The results of this project show that as bacteriophages are exposed to more ultraviolet light they reproduce less. In the experiments done with the bacteriophage concentration 10^7 there was a dramatic decline in phage reproduction. The sample of the concentration 10^7 that was exposed to 10 sec of U.V light had 33 plaques while the sample that was exposed to 40 sec of U.V light only had 7 plaques. In comparison with the control which had 35 plaques the 10^7 with 40 sec of exposure only had a 20 percent rate of growth. The concentration 10^4 had a similar reaction to the U.V light as the 10^7. The concentration 10^4 after 10 seconds of ultraviolet exposure produced 15 plaques. The number of plaques produced after 20 seconds was only 7 meaning that phage reproduction was cut nearly in half. There is a large discrepancy between the number of plaques formed with the concentration 10^7 that was exposed to 10 sec of U.V light and the concentration 10^4 that was exposed to 10 sec of U.V light. This discrepancy is due to the difference in viral cell concentrations. The concentration 10^4 has fewer viral cells to reproduce with than the concentration 10^7. This leads to fewer new viruses being created and fewer plaques.

Data Reduction of Bacteriophage Lytic Activity From U.V Exposure					
Seconds	***10^{-5}***	***10^{7}***	***10^{4}***	***10^{1}***	***10^{-2}***
Control	***35***				
10		33	15	1	0
20		22	7	0	
30		12	6		2
40		7		0	0



Conclusions

This project has shown that prolonged exposure to ultraviolet light decreases bacteriophage reproduction. This information is of consequence for ultraviolet light sterilization. The reason this is of consequence is because it shows how much ultraviolet light exposure is necessary to kill viruses at different concentrations. This research can tell how much ultraviolet light exposure is needed to sterilize a certain amount of water with certain viral cell concentrations. This could help with things like water sterilization procedures that use ultraviolet light. There is one validity issues that has come up with this project. The issue is that there was only one control done in this project. The control was the phage concentration 10^{-5}v.c per ml that was exposed to no U.V light. This was to show what normal phage growth would be like but since it was not done with any of the concentrations used in the experiments an accurate comparison between normal phage growth and growth of phage after U.V exposure can not be made.

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