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UV Irradiation on Bacteriophage Survival

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Abstract

Bacteriophage is of great interest because of its potential role in controlling bacterial populations in our environment. UV exposure has a damaging effect on the virus decreasing lytic ability. This study set out to test the effects of UV radiation, in amounts comparable to local environmental conditions, on bacteriophage T2. The virus was placed in a Petri dish in a PBS medium and exposed to UV radiation at 365nm. The irradiated virus was allowed to infect E. coli and plated. The plaques formed were counted to determine lytic activity of the virus with respect to UV irradiation. The results showed that lytic activity decreased by about the same amount after 15 – 30 minutes, but after that showed a steady decrease as exposure time increased.

Introduction

Bacteriophages are viruses that infect bacteria. This involves a host-specific parasitic relationship in which the phage uses the energy and the metabolic machinery of a bacterium to produce more phage. The bacterium is eventually destroyed and the phage particles are released, able to infect the surrounding bacteria.

Structurally a phage consists of a nucleic acid genome that is contained within a protein capsid. Some virions (the basic viral unit) also have a lipid or a proteinaceous tail (DePaepe and Taddei, 2006). Most phages are tailed and contain double-stranded DNA as their nucleic acid, This type belongs to the order Caudovirales, to which over 95% of known phages are classified (Maniloff and Ackermann, 1998). The three main families of phage are differentiated by morphological characteristics. Myoviridae phages, including many which infect members of Enterobacteriaceae (Goodridge et al. 2003), have double-layered contractile tails, Siphoviridae phages have long flexible tails and Podoviriae have short stubby tails.(Deveau et al. 2006). There are considerably fewer members in the ten small families of tailless phages, which are characterized by shape, segmentation, whether the DNA or RNA genome is double- or single-stranded, and whether or not the phage is enveloped in a lipid coat (Kutter and Sulakvelidze, 2004).

Bacteriophages display two possible lifecycles: virulent or temperate. Virulent bacteriophages quickly cause lysis, or cell destruction, and rapidly kill bacterial cells. The timing of this action is carefully controlled, because if lysis occurs too quickly, not enough new phages will be produced for infecting new bacteria cells. If too late, the phage loses an opportunity for infection of new host cells and further replication (Kutter and Sulakvelidze, 2004). Temperate viruses typically integrate their DNA into the host bacteria's. The prophage, or latent form of bacteriophage, has a circular form of the phage's genome. Alan Campbell postulated that the bacteriophage physically inserts its genome into the host genome, forming the circular prophage genome. Then there is crossing over between the prophage genome and the circular bacterial genome (Kutter and Sulakvelidze, 2004). This allows it to replicate at the same time as the bacteria (Hanlon 2007). The phage's genome in this state is called a prophage, and during this time, the bacteria may appear to be dividing in phage-free conditions (Kutter and Sulakvelidze, 2004). It will remain integrated until it is induced by adverse conditions such as exposure to UV light and then proceed to cause lysis (Kutter and Sulakvelidze, 2004).

One of the more studied and well-known supergroups of bacteriophage is the T7 group, which is made up of thirteen fully sequenced phages. In a study on T7-like phages, closely related phages were found in different countries, which leads to speculation that gene transfer is horizontal and that there is less global diversity than there is local diversity (Ceyssnes et al. 2006). However, another study concluded that strongly lytic phages, with a unique and well-organized replication strategy, show less inclination to participate in horizontal gene transfer. This is thought to be because the lifecycle of strongly lytic phages proceeds so swiftly, that the tendency is to evolve largely by speciation, accumulation of point mutations and genetic

adaptations (Kovalyova et al. 2003). This study uses a member of this group, the T2 bacteriophage.

Bacteriophages are ubiquitous in nature and are claimed to be the most abundant living entities on the earth (Kutter and Sulakvelidze, 2004). Several studies have sought to study and compare characteristics of bacteriophage from soil and water of various environments. Ashelford (2003) studied methods for finding the total number of bacteriophage in soil and found that in some cases, the bacteriophage population exceeded that of the bacteria. Previous work on bacteriophage and virus-like particles found in soil has shown that viral adsorption to the host cell is influenced by the characteristics of the soil solution such as; ionic strength and composition, pH, the presence of dissolved organic matter and soil features such as water content, the presence of organic coatings, and clay and organic matter content. Also important are characteristics of the virus itself, such as hydrophobicity and the isoelectric point. Analysis revealed that there was a significant correlation between virus abundance and water content of the soil. Wetland soil samples showed the most overall diversity (Williamson et al. 2005). Viruses and bacteria have been found to be most plentiful in moist soils that are rich in organic matter. It was found that in temperate soils, the bacteria and phage populations were more dependent on each other. This indicates that virus production is not a slow and steady process, but one that adapts quickly and responds to changes in host growth (Srinivasiah et al. 2008).

Bacteriophage have also been isolated from water. In a study in Hawaii, researchers found that viruses are greatly responsible for deaths of bacterioplankton and phytoplankton, indicating the major role that viruses play in a marine environment's microbial ecosystem. Marine phages from Hawaii, while unique, share many similar characteristics with other phages isolated from marine environments (Jiang et al. 1998). Virus populations from marine

environments are greatly increased (as much as 2000 times) compared to populations in wetlands and agricultural soils. Other studies have shown that in productive coastal environments, such as coastal South Carolina, double stranded DNA virus populations turn over once or twice a day and destroy a significant amount of bacteria (Srinivasiah et al. 2008).

Bacteriophages are also studied because of the myriad applications to medicine. A recent study published by Merabishvili et al. (2009) helped to create a standard for phage therapy in human patients, with clear laboratory procedures for creating a well-defined bacteriophage treatment. Patients with burn wounds received this treatment to combat bacteria commonly known in burn hospitals, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The patients experienced no adverse effects from the treatment, while the specific interaction of the phage with the target bacteria, reducing infection, was confirmed using transmission electron microscopy.

Bacteriophages also hold medicinal value because of their ability to infect bacteria that have grown resistant to antibiotics. In addition, for use in therapy, a phage can be engineered to destroy bacteria more efficiently and they can be modified to be nonreplicative, reducing the risk of leaving lysogenic particles in patients (Lu et al. 2009). Antibiotics are generally ineffective against biofilms, a thin layer consisting of a polysaccharide film interspersed with colonies of bacteria. The biofilm can insulate the bacteria against the usual methods of disinfection or treatment regimes. Biofilms can lead to problems with infection and contamination in medical and food processing settings. In a study by Lu et al. (2007), engineered bacteriophage were seen to effectively remove biofilms.

Phage therapy is gaining popularity, but still faces problems, such as lack of clinical trials and the release of toxins when cells are lysed. The specificity of each phage has both advantages

and disadvantages. Phage will not infect human cells or harmless bacteria, but to create a treatment, the bacteriophage will have to be genetically engineered for specific situations (Lu et al. 2007).

There are several typical methods for characterizing bacteriophage. First, the phage spot test on bacterial lawns of different strains allows for the selection of the phage infecting the most strains of a particular type of bacteriophage. The morphology of the phage is another common method, involving the use of transmission electron microscopy. The size of the genome can be used as well, determined through application of restriction enzymes. Last, SDS-PAGE can be used to analyze protein composition (Sillankorva et al., 2008). Phages can also be classified based on the conservation of gene arrangement, but the phage must be completely sequenced for this (Kovalyova and Kropinski, 2003).

The biggest challenge in studying phage is understanding the replication of the virus's genetic material. Many studies use UV to study phage reproduction because evidence suggests it is closely linked with this process (Bowen, 1953). Some studies see survival of phages after exposure to UV as an indication that a mechanism of biological repair has been activated and carried out (Chiange and Harm, 1974). Other studies have used UV to look at the enzyme-substrate complex and reaction rates in reactions involving phage DNA (Evdokimov et al., 2007). In addition to this useful aspect, UV can also be used to show what effect stress has on phage growth. Phage found in the environment is not likely to be growing under the optimal conditions that can be found in a lab (Ellis and Delbruck, 1939). Because of stress, phage may have less impact on bacterial populations and more trouble replicating. Using UV is one way to look at phage under stressful conditions. We will be looking at the effect that differing amounts of UV radiation has on phages. That is, we will expose phage for varying lengths of time to UV

radiation and allow it to infect bacteria, comparing the amount of bacteria the phage can destroy to the amount of bacteria destroyed when unexposed phage is used. After determining what assay works best to show the reduction of phage and at what concentration the effect is best viewed, the study can move into gel electrophoresis of proteins in UV-damaged bacteriophage.

Methods

Commercially bought phage was diluted using PBS to concentrations of 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}. Sample trials suggested the most effective results would be seen at 10^{-3}, so all future trials were run at that concentration. Four 100-ul drops of virus were placed close together on an empty Petri dish and placed under the UV lamp. The long wave filter was selected (365 nm), and every 15 minutes, one drop was removed and placed into a microcentrifuge tube along with 400 ul of E.coli. The tubes were mixed well and kept at 37°C for 10 minutes. After incubation, 250 ul of the virus and bacteria mixture was added to 11 ml of warm top agar and poured onto a Petri dish. The dishes incubated overnight, and the number of clear zones on the plate where virus had killed bacteria were counted. Top agar was made with 0.9g agarose, 3.75g LB broth, and 150 ml DI water. It is less dense than normal agar and allowed a better look at the growth of virus and bacteria on a plate.

Results and Discussion

The number of plaques were counted for each length of time and compared to the control group corresponding to that trial. The averages were taken and plotted as percentages in Figure 1. As seen in the plot, the control group had a significantly higher survival rate than any of the groups that were exposed to UV. Comparing plaque count of irradiated phage to non-irradiated phage showed that lytic activity decreased with increased exposure to UV. There was a small difference in lytic activity between the 15- and 30-minute trials, but after that, a clear downward

trend is seen in groups exposed for longer than 30 minutes. Table 2 shows the calculated standard deviations of each time segment. A problem with the raw data was that many of the plates showed smears of cleared space (mimicking lytic activity) where individual plaques were not able to be counted. The smears ranged from very thin and short; to long and wide, while a few covered almost ¼ of the dish. This smear effect could be lessened by decreasing the concentration of phage by one half, to 5E-4. Since individual plaques are not being counted, the average percentages may be lower than in actuality.

	1	2	3	4	5	6	7	8	9	10	11	12	Average	% of Control
Control	102	92	169	148	144	150	174	156	185	165	181	124	149.1667 SD 29.79	100
15 min	145	78	143	116	90	62	153	139	137	150	122	108	120.25 SD 30.13	80.61453
30 min	150	93	86	92	125	131	147	106	122	138	107	104	116.75 SD 21.88	78.26816
45 min	96	67	113	83	92	110	115	86	116	123	95	113	100.75 SD 16.84	67.5419
60 min	68	47	87	66	89	121	88	69	115	78	81	102	84.25 SD 21.15	56.48045

Table 1. Plaque counts were averaged and compared to non-irradiated phage plaque counts.

	Average Plaque Count	% of Control	Standard Deviation
Control	149.1667	100	29.78661
15 min	120.25	80.61453	30.12738
30 min	116.75	78.26816	21.88036
45 min	100.75	67.5419	16.84218
60 min	84.25	56.48045	21.15366

Table 2. Standard deviation was calculated and used to describe error in percentage comparisons.

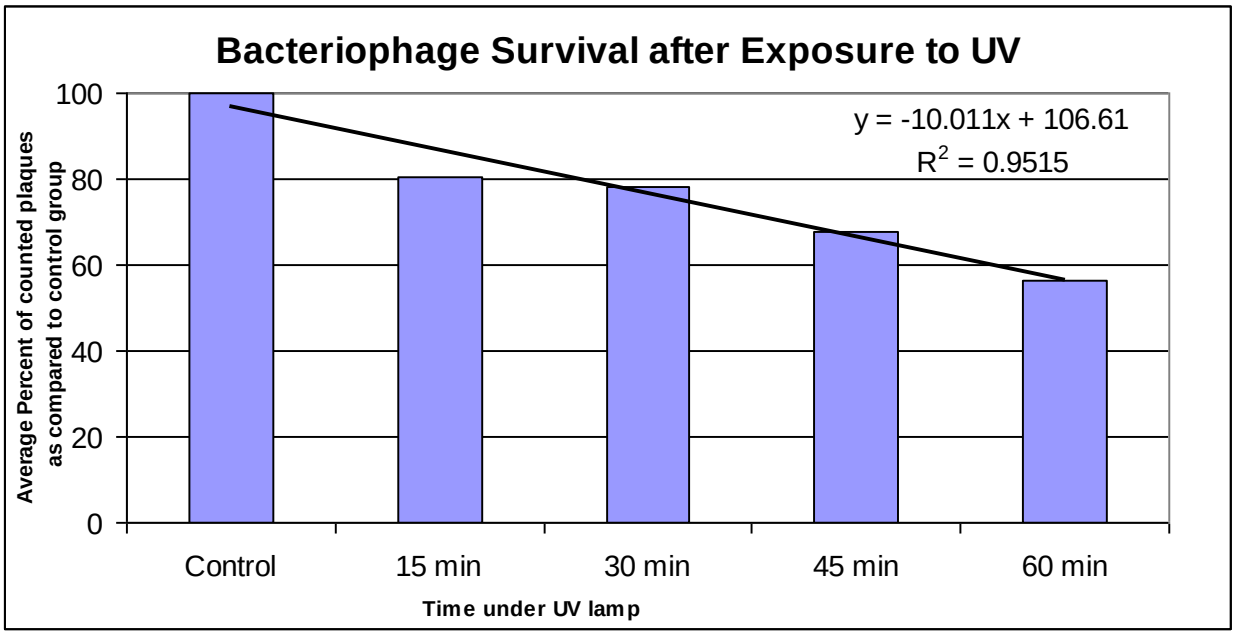

Figure 1. The number of clear areas on the bacteria caused by irradiated virus were counted and compared to the amount counted for the control group. The averages were taken and plotted as a bar graph in order to show the trend of the data as the virus was exposed for longer times to the UV.

Conclusion

The trend seen in the results followed what was expected. Over time, lytic activity decreased, falling in a steady decline for exposure times greater than 30 minutes. After 15 minutes, lytic activity dropped to 80.6 ± 30.1% of non-irradiated phage. After 30 minutes, activity was 78.27 ± 21.88%; after 45 minutes, 67.54 ± 16.84%, and after 60 minutes, activity fell to 56.48 ± 21.15%. The high margin of error emphasizes the large differences in plaque counts from trial to trial seen in Table 1. With more repeated trials, the error would grow smaller and the averages would approach the true values showing the trend of lytic activity decline over periods of exposure to UV. The most difficulty came from the irregularity in clear areas, namely the streaks where individual plaques were not distinguishable. This could be fixed in future by varying the concentration of the virus exposed to UV and plated. In this study, because assay techniques did not become successful until the last few weeks of available research time, it was not feasible to search further for a concentration producing more conclusive results. Another issue with this study was that it did not mimic many environmental conditions and all UV exposure was done for virus in PBS in a Petri dish. The lab setup was only similar to direct exposure in an open field. In the future, this study would mimic other environmental conditions, such as phage existing in soil and murky water, at various depths, to better understand UV's effect on bacteriophage survival. This study intended to look at the protein expression patterns of bacteriophage with respect to UV conditions, so that can also be included in future study.

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