222 nm UV Disinfection of Bacteriophages as Surrogates for Eukaryotic Viruses

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Abstract

Introduction
Germicidal ultraviolet (GUV) radiation, particularly shortwave UV-C (200-280 nm) is a promising technique used for disinfection of several microbiological targets. In the wake of the COVID-19 pandemic, its use for disinfecting eukaryotic viruses has garnered attention as an intervention to prevent transmission of airborne respiratory viruses.

Purpose and Methods
This study examines 222 nm GUV, primarily in liquid, and uses various bacteriophages (MS2, Phi6, T1, and T4) as surrogates to model eukaryotic viruses, aiming to broaden current understanding of GUV disinfection efficacy and virus models. Usage of bacteriophages (RG1 organisms) as virus surrogates serves as a low risk alternative to handling pathogenic viruses (RG2 organisms), allowing for safer infectious disease transmission mitigation research. UV exposures of bacteriophages were conducted in a collimated beam chamber designed according to Bolton and Linden (2003) [1], which was validated using iodide/iodate actinometry.

Findings
For MS2 phage experiments, approximately 0.75, 1.1, 4.5, and 6.4 log reductions in PFU/mL were observed following UV exposures of 7.60, 15.22, 45.65, and 91.29 mJ/cm^2, respectively. Liquid experiments with other phages are still ongoing.

Background

- Shorter 222 nm UV radiation does not result in damage to the skin and eyes of mice and rats, which 254 nm UV is known to cause [2,3].
- Influenza viruses are most susceptible to UV radiation in the range of 200-230 nm [4].

Virus	Size	Structure
MS2 Phage [5]	3569 bp, 25 nm	tail-less, unenveloped, linear ssRNA
Phi6 Phage [5]	13385 bp, 85 nm	tail-less, enveloped, segmented dsRNA
Influenza (H1N1) [5]	13588 bp, 80-120 nm	tail-less, enveloped, segmented ssRNA
SARS-CoV-2 [6]	26-32 kb, 80-120 nm	tail-less, enveloped, linear ssRNA

Objectives and Methods

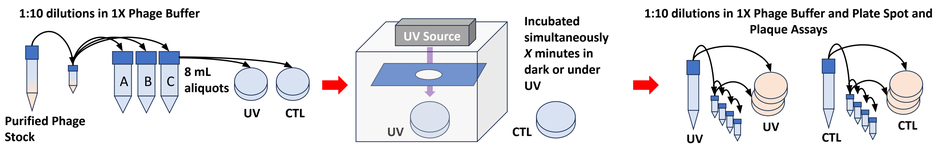

Figure 1. Objectives: To (1) use various bacteriophages as eukaryotic virus models, (2) in order to determine the effectiveness of 222 nm germicidal UV light as a method for viral disinfection, (3) by quantifying phage survival after UV exposure in liquid in PFU/mL.

Methods: Purified phage stock was diluted and exposed to 222 nm UV in 8 mL aliquots, before collection, further serial dilution, spotting onto plates with bacterial host overlays, and incubation. Following spot assay results, plaque assays were performed with the same samples for more accurate phage concentration quantification.

Results

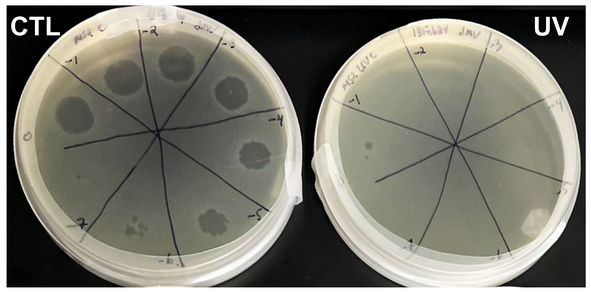

Figure 2. MS2 spot assay for a 30 minute UV exposure
- Control: 3.33E+09 PFU/mL; UV: 6.67E+02 PFU/mL
- Phage concentration reduction of 6.7 log

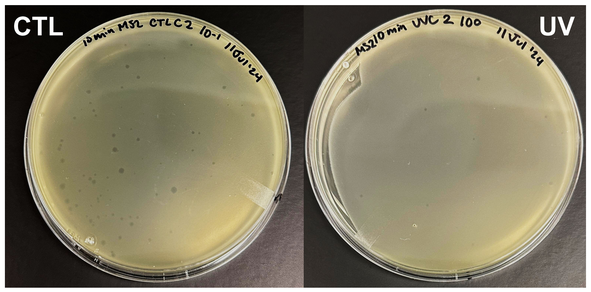

Figure 3. MS2 plaque assay for a 10 minute UV exposure
- Control: 2.5E+05 PFU/mL; UV: 4.0E+03 PFU/mL
- Phage concentration reduction of 1.8 log

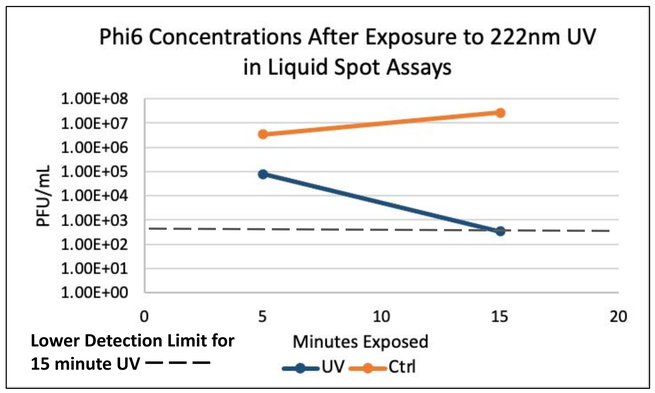

Figure 4. Preliminary Phi6 concentration reductions from spot assays
- 3.33E+06 to 7.78E+04 PFU/mL after 5 minutes
- 2.67E+07 to 3.33E+02 PFU/mL after 15 minutes

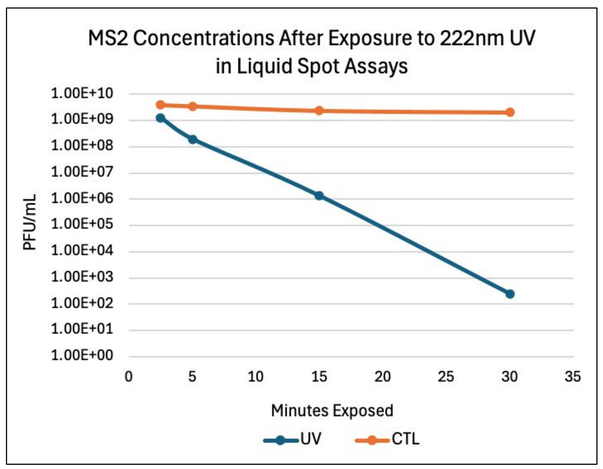

Figure 5. Average MS2 concentration reductions from spot assays
- 3.92E+09 to 1.25E+09 PFU/mL after 2.5 minutes
- 3.50E+09 to 1.92E+08 PFU/mL after 5 minutes
- 2.33E+09 to 1.33E+06 PFU/mL after 15 minutes
- 2.00E+09 to 2.50E+02 PFU/mL after 30 minutes

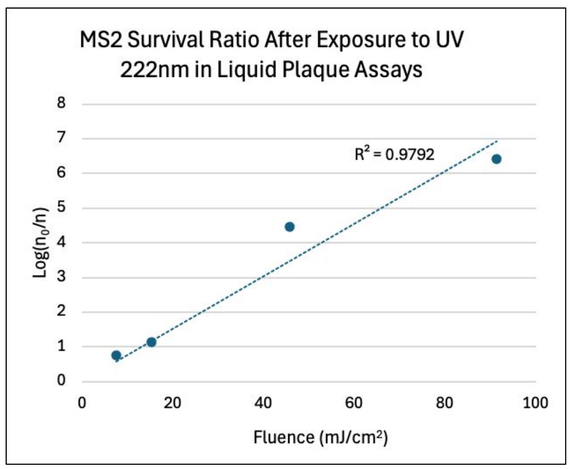

Figure 6. MS2 plaque assays
- Log reductions in phage survival ratio (n_0/n) of 0.75, 1.1, 4.5, and 6.4, following 2.5, 5, 15, and 30 minute exposures.
- Respective percent reductions were 82.15%, 92.56%, 99.997%, and 99.99996%.

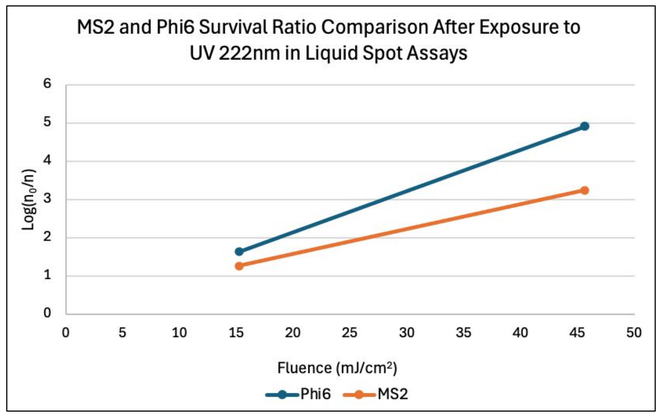

Figure 7. Comparison of MS2 and preliminary Phi6 survival ratio (n_0/n) reductions from spot assays
- Phi6: 1.63 log; MS2: 1.26 log after 5 minutes
- Phi6 4.90 log; MS2: 3.24 log after 15 minutes

Conclusions

- 222 nm UV exposures in liquid effectively reduces MS2 and Phi6 survival.
- Increasing UV dose (fluence) leads to a higher rate of phage disinfection.
- Preliminary spot assays indicate that Phi6 is more susceptible to disinfection by 222 nm UV than MS2, as seen by increased log reduction in survival following the same amount of UV exposure.

Future Directions

Ongoing experiments in liquid with Phi6 will continue, followed by T1 and T4 phages. UV exposure time intervals will be expanded for liquid experiments with all phages, aiming to span the growth curve of all phages. Planned exposure times to be added are 7.5, 10, 20, and 45 minutes.

In addition to survival quantification in PFU/mL, genomic assays will be completed to understand the effect of UV exposure on the genome of bacteriophages. Both nucleic acid extractions and digital PCR will be performed.

In collaboration with CITY@UMD, the effect of 222 nm UV will be assessed in an aerosol state via a single pass flow chamber. All experiments will also be repeated using target eukaryotic viruses, including influenza, to compare virus and phage behavior.

References

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