Demonstration of nucleic acid repair in viruses after UV disinfection using coliphages as surrogates

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Motivation

- Improve water treatment technology
- Develop understanding of high levels of resistance for certain waterborne pathogens

Background

- Germicidal wavelength: 200-300 nm
- Peak DNA absorption at 260 nm
- Photolyase: photo-repairing enzyme
  - Activated at wavelengths 350-450 nm

Objectives

- Determine possible reactivation of viruses utilizing host photorepair mechanisms
- Determine if MP lamps (polychromatic) are more effective than LP lamps (monochromatic) at inactivating phages.

Background ~ UV damage and repair

- Low pressure mercury vapor lamp (monochromatic light)
  - 254 nm wavelength
- Medium pressure mercury vapor lamp (polychromatic light)
  - 200-600 nm wavelengths
**Experimental process**

- **Phase 1**: Determine dose response for each of the three bacterial hosts under the LP UV lamp (Famp, CN13, & LT2).
- **Phase 2**: Confirm photo-repair ability and parameters of bacteria after exposure to LP UV lamp.
- **Phase 3**: Determine dose response for each phage (T1, PRD-1, PHIX174) under both LP and MP UV lamps.
- **Phase 4**: Demonstrate reactivation of virus using host repair mechanisms after exposure to LP and MP UV lamps.

**Phage and Host**

- **Phage**
  - PRD-1: double-stranded DNA
  - T1: double-stranded DNA
  - phiX174: single stranded DNA

- **Bacteria**
  - LT2 (salmonella)
  - CN13 (E.Coli)

**Results**

- Bacteria dose-response

**Photo repair shop**

- Low pressure UV collimated beam system

**Bacteria Photorepair**
Maximum photoreactivation of the bacterial host after UV exposure

- Salmonella (LT2) 3 hrs
- E coli (Famp) 2hr
- E coli (CN13) 1 hr

Phage Dose-response

Spot plating

- Saved time and equipment
- 5 dilutions per plate opposed to three plates per dilution

Comparison to Double agar layer method:
1st trial: DAM ~ 3.3 x 10^4
2nd trial: DAM ~ 5.1 x 10^9
Spot test: ~ 4 x 10^7
Spot test: ~ 4.8 x 10^9

Phage repair using host photo-repair mechanism

Phage Repair Experiment

Initial Concentration
Concentration after LP exposure ~ 40 myc/ml
Concentration after 6 hours of light exposure
Concentration after 6 hours of dark
Phage repair

- 6 hours under photo-repair lamp

**Conclusion**

- Phage with double-stranded DNA were able to repair using bacterial host repair mechanisms, but phage with single stranded DNA were not.
- More experiments need to be run to determine if MP lamps are more effective at inactivating PRD-1, T1, and phiX174.

**Future work**

- Develop dose response curves for Photoreactivation of phage.
- Further investigate efficacy of Medium pressure lamps as opposed to Low pressure lamps.

**Questions?**
References

Rocky Mountain Laboratories, NIAID, NIH