Guide: Sutirth Dey

Saumil Shah

20151179

Effects of Ultraviolet Irradiation on *Escherichia coli* at Different Phases of Growth

Spring 2018

Mid Semester Report

# Introduction

Microbes, which involve algae, archaea, bacteria, fungi, protozoa and viruses, are tiny forms of life that surround us. Albeit some microbes are beneficial to us, most of them are pathogenic and can be fatal at times. These pathogens show a diverse fashion in infecting host organisms. Nevertheless, most of this is through the air, a lot of them also propagate through contaminated surfaces which come in contact with hosts along with contaminated food and water.

Over the past few decades, ultraviolet germicidal irradiation (UVGI) has become extremely popular as a disinfectant since it works on almost all kinds of microbes. UVGI has a wider range of application including wastewater treatment, air - water - food disinfectant along with medical and laboratory hygiene. Compared to other chemicals this method is very favorable in terms of cost and labor.

Although UVGI is becoming a paramount anti-infective approach, very less quantitative data is available on radiation susceptibilities of target microbes. More interestingly, even lesser work has been done on side effects of this method. Radiation dosages which are not lethal can cause extensive photochemical changes in microbes. However, DNA is the primary target for the alterations because it is the largest molecule in the cell. Also, DNA can absorb ultraviolet radiation very efficiently, in addition to that, compared to RNA, DNA is present in fewest copies. In some cases, as a result of this modifications, resistance to various stresses may arise. Antibiotic resistance develops typically as a spontaneous mutation (Demerec, 1948). With overuse of antibiotics in healthcare and agricultural fields, antibiotic resistance has increased dramatically over past several decades which is of prime concern.

To develop a better understanding and preventive strategies, it is essential to study the direct and indirect responses to ultraviolet radiation. In real life scenarios, microbial cultures are not synchronized. Hence they may get exposed to ultraviolet at different phases of growth this may give rise to unforeseeable changes in microbes. Keeping these objectives in mind, a study is being conducted on *Escherichia coli*.

# Aim

To investigate correlated changes in *Escherichia coli* due to ultraviolet irradiance at different phases of growth.

# Objectives

1. To select the *E. coli* populations for ultraviolet resistance.
2. To investigate direct responses of *E. coli* to ultraviolet irradiation at different phases of growth.
3. To investigate effects on fitness and survivability of selected *E. coli* population.

# Methods

## Bacterial Strain, Media & Culture Conditions

*E. coli* strain MG1655 was used for the experiments. The stock cultures of *E. coli* were revived on either solid media plates or in liquid media as per requirements of the experiments. Selection and Standardization experiments were carried out in liquid broth.

Nutrient Agar (NA) was used for all the solid cultures and Nutrient Broth (NB) was used for all the liquid cultures, both contained 50 µg/ml of Kanamycin as a selective component for the MG1655 strain. Media and working solutions were autoclaved for 20 minutes at 121 °C and 15 PSI pressure for sterility. All cultures were incubated in the dark with constant shaking at 150 rpm and temperature of 37 °C unless mentioned otherwise.

Different populations were maintained for the experiments labeled as **L**ag, **E**xponential, **S**tationary, **C**ontrol, **A**ncestor, **Co**mbination, **U**npredictable, **P**redictable and **Er**ythromycin (LESCA CoUPEr). Stocks were prepared and stored after 10 cycles of selection and growth.

## Selection

A Phillips 8 watt TUV ultraviolet lamp was used to simulate ultraviolet radiation. **LE** populations were radiated at lag and exponential phase, respectively, and **SCoUP** populations were radiated at stationary phase before subculturing by an ultraviolet lamp. **C** and **Er** populations were subcultured in NB and NB + Erythromycin, respectively, without any exposure to ultraviolet. Ancestors were used directly for assays without any subculturing.

## Standardization

To test the correlated effects of ultraviolet exposure, the survivability of the selected populations will be tested in a number of stressful environments. For this survivability assays, the sub-lethal concentrations were standardized. The sub-lethal zone was defined as 50-70% aggregated inhibition in Growth Rate and Carrying Capacity. A wide range of concentrations were used for the first round of standardization followed by a narrow range second round.

Controls for all the standardization experiments were grown in NB. pH stress was simulated by adding Hydrochloric Acid (HCl) and Sodium Hydroxide (NaOH) to the broth. 30% Hydrogen Peroxide (H2O2) was used to imitate peroxide stress. Chloride salts of Iron (FeCl­3), Cobalt (CoCl2), Nickel (NiCl2) and Copper (CuCl2) were added to mimic heavy metal stress. Tincture of Iodine, Phenol and Sodium hypochlorite were used as a disinfectant group. Erythromycin, Ampicillin, Chloramphenicol, Norfloxacin and Rifampicin were added to NB for antibiotic stress.

## Growth Curve

Bacterial growth was proxied by Optical Density (OD). The assays were conducted in 24 well tissue culture plates with measuring OD every 20 minutes at a wavelength of 600 nm using a microplate reader. Obtained data was then analyzed for growth rate, carrying capacity and doubling time.

# ReSULTS

Standardized values of sub-lethal concentrations of some stresses are as follows

|  |  |
| --- | --- |
| Stress Condition | Concentration |
| Ultraviolet Exposure | 15 seconds |
| Erythromycin | 15 µg/ml |
| Cobalt(II) chloride | 100 µg/ml |
| Nickel(II) chloride | 150 µg/ml |
| Copper(II) chloride | 100 µg/ml |

# Future Directions

Numerous stress environments inspired by realistic situations, which bacteria may face, like Chlorhexidine (Disinfectant), Allicin (Garlic) and Azadirachtin (Neem) can also be included.

After every 10 cycles of selection and growth survivability of each of the populations will be checked and compared with Ancestors.

# References

[1] Demerec, M. (1948). Origin of Bacterial Resistance to Antibiotics. *Journal of Bacteriology*, 63-74.

[2] Kendric, S. (2008, September 17). *Basic Ultraviolet Radiation Photobiology*. Retrieved from Photobiological Sciences: http://photobiology.info/UVphoto.html

[3] Saz, A., Eagle, H., & Toal, J. (1952). The effect of ultraviolet irradiation on the development of resistance of bacteria to antibiotics. *Journal of Bacteriology*, 513-23.