The Use of Bactericidal Ultraviolet Radiation in the Eradication of *Escherichia coli* K12

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ABSTRACT: For half a century, ultraviolet (UV) radiation has been a common method of sterilization in fields such as food manufacturing, water sterilization, and hospital surface disinfection. To further explore UV radiation's effectiveness as a germicidal agent, my research focused on the irradiation of *Escherichia coli* (*E. coli*). A two-stage study was conducted where the effects of irradiation before and after incubation of the bacteria were measured to test the sterilization efficiency of UV radiation. The non-shiga toxin producing K12 *E. coli* strain was used instead of the more dangerous O156:H7 strain—however, they have similar structural dexterities and reproductive patterns. Based on these trials, the results were inconclusive despite showing consistent decrease in colony sizes because the differences in size were not statistically significant. In the most successful trial (Trial 2; irradiation pre-incubation), the maximum difference in survival was about 40% lower in the experimental group than the control. The problems with data validity were partially due to limitations in available equipment and methods in a high school environment.

INTRODUCTION

Escherichia coli (*E. coli*) is a relatively harmless strain of bacteria found in the colon of many warm-blooded organisms. A particular strain, *E. coli* O156:H7, produces a powerful Shiga toxin and can cause diarrhea, abdominal pains, and kidney failure if ingested. The bacteria strain exists in raw or undercooked foods. It is prevalent in raw beef due to the bacteria's natural cultivation on cattle farms.[1] Prolonged exposure to ultraviolet (UV) radiation at a wavelength of 254 nanometers (nm) kills foodborne pathogens such as *E. coli* without affecting the quality of the meat.[2] This is due to DNA mutations that occur when UV light is absorbed by deoxyribose molecules of prokaryotic bacteria and viruses.[2] UV radiation has been a part of food sterilization for over 50 years; grocers treat produce, beverages, and cheeses with UV light throughout the production process.[3] However, many people are skeptical about the effects of UV radiation on food quality (organic food legally cannot be irradiated) as well as the cost of such radiation treatments.

Radiation is a popular means of disinfecting water, food contact surfaces, medical instruments and surfaces, and ensuring sterile processing of popular grocery items such as cider, juice, produce, cheeses, and egg products.[4] Despite this, many people find the concept of food radiation sterilization unsettling. Radiation's negative connotation hinders the use of radiation in the food production process. Although UV irradiation is a proven method of prolonging shelf life and ensuring consumer safety, skeptics of radiation as well as high costs and federal regulations affects companies from implementing iradiation treatments.

RESULTS AND DISCUSSION

We evaluated the effectiveness of UV-C irradiation on cultures of *E. coli* grown in nutrient-rich agar under several environmental conditions to simulate the different stages of the farm-to-table process. We tested the effects of UV irradiation pre- and post-incubation of the bacteria. Due to limitations in material availability, the tests were limited to the non-Shiga toxin-producing K-12 strain of *E. coli* in an *in vitro* experiment as opposed to the preferred method of culturing the ground beef O156:H7 strain. Our research was also limited to hand-held UVC-3 3-watt DC surface sterilizers as opposed to industrial grade sterilizers that perform at 40 watts.[5]

The radiation's destructive effects are shown by the decrease in colony density relative to the control which continued to increase in size. However, given that an estimated area was being compared to another estimated area, there is no statistical test to compare our data and therefore no statistical significance in our data. In future studies, this will be fixed via more trials and larger sample sizes.

The samples were incubated at 37°C and had an approximate area decrease of 9.74% while the control area increased by 6.4%. At 23°C, there was about 14.26% decrease in area while the control increased by 3.37%. There was no growth in the petri dishes incubated at 5°C. Although 5°C is within the temperature range where *E. coli* can survive, the colder temperature slows the bacterial metabolic processes and inhibits reproduction. The refrigeration temperature was adjusted accordingly to 10°C beginning in trial 2.

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Table 1: Results from Trial 1 (Bottom half is control)

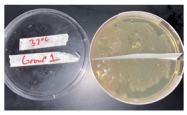
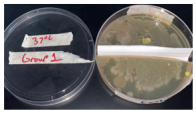
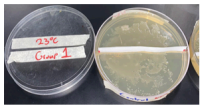
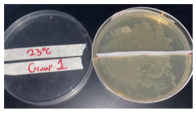
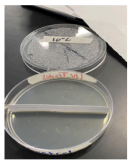
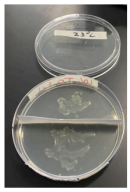
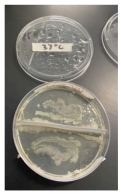
Day 1 - 37°C Post- 72-hour incubation period prior to irradiation Average Control Radius: 3.0cm Average UV Radius: 4.0cm Initial Estimated Area: Control: 14.14 cm^2 UV: 25.13 cm^2 	Day 5 - 37°C After 10 irradiation sessions Average Control Radius: 3.1cm Average UV Radius: 3.8cm Final Estimated Area: Control: 15.10 cm^2 → +6.4% UV: 22.68cm^2 → -9.74% 
Day 1 – 23 °C Post- 72-hour incubation period prior to irradiation Average Control Radius: 2.9cm Average UV Radius: 2.7cm Initial Estimated Area: Control: 13.21 cm^2 UV: 11.45 cm^2 	Day 5 – 23 °C After 10 irradiation sessions Average Control Radius: 2.95cm Average UV Radius: 2.5cm Final Estimated Area: Control:13.67cm^2 → +3.37% UV: 9.82cm^2 → -14.26% 
Day 1 - 5°C Post- 72-hour incubation period prior to irradiation **NO GROWTH**	Day 5 - 5°C After 10 irradiation sessions **NO GROWTH**

Table 2: Results from Trial 2 (Bottom half is control)

(10°C) No Growth	
(23°C) Average Control Radius: 1.8cm Estimated Area: 10.18 cm^2 Average UV Radius: 1.4 cm Estimated Area: 6.16 cm^2 Difference: 39.49%	
(37°C) Average Control Radius: 2.1cm Estimated Area: 13.85 cm^2 Average UV Radius: 2.0cm Estimated Area: 12.57 cm^2 Difference: 9.24%	

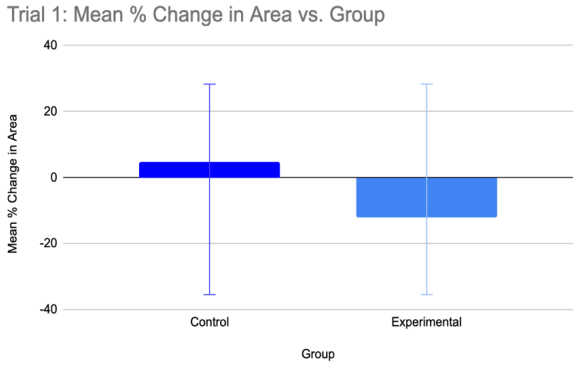


Figure 1. The graph portrays the resulting mean percent changes of the control and experimental groups of the first trial. The graph shows clearly that there is no statistical difference between the two means as the standard error bars of both overlap. These results led to a conclusion of no statistical significance.

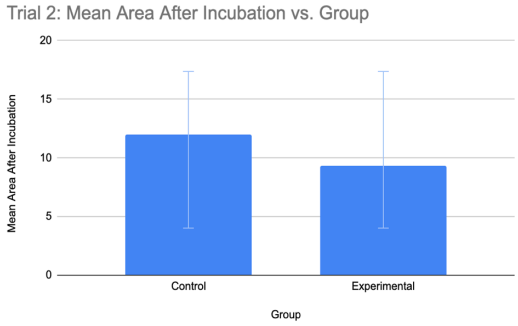


Figure 2. The graph for the results of trial two represents the mean areas of the control and experimental groups following the irradiations sessions and incubation. The graph does show a small difference in mean area between the two groups, but due to the small sample size the standard error bars overlap proving no statistical significance.

During the first trial, UV radiation was relatively ineffective against *E. coli* colonies when irradiated post-incubation. Despite the difference in percent change between the control and experimental groups, a t-test showed the results were not statistically significant. Given the large standard deviations visible in the bar chart as overlapping error bars (the control was 2.1425 and the experimental was 3.1961), there is not enough evidence to support a statistically significant difference. Although the experiment showed a clear difference between the control and variable groups, the percent change is not large enough to consider UV light effective in the destruction of already existing colonies. This is likely due to UV light being germicidal. UV radiation damages bacterial DNA, which prevents the bacteria from replicating and effectively neutralizes it; when a pre-existing colony is irradiated a mac-

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roscopic observation will show little decrease in density, but further reproduction will not occur.

It was found during the second trial that UV radiation has an obvious effect on the reproductive efficiency of the *E. coli*. However, the results were far from the 99% difference between the control and experimental treatments advertised on the lamp packaging. A t-test showed large standard deviations (Control: 2.5951 and Experimental: 4.5326) that overlap as error bars. These large standard deviations are a thought to be a result of the inability to collect more data; one Petri dish for each temperature within the control and experimental groups is not enough to show statistically significant results.

It was found that the refrigerated sample, even at 10˚C, prevented reproduction. The sample incubated at 37˚C produced only a 9.24% difference between the control and irradiated side, while the room temperature sample experienced a difference of 39.49%. These differences are reasonable given that *E. coli* is more likely to thrive in an environment similar to that of the human colon.

The second trial focused on using radiation to prevent the reproduction and colonization of *E. coli* rather than destroying existing colonies. The test was set up with three dishes divided in half with cardstock and exposed to the bacteria. One side of each dish acted as the control and the other was exposed to UV radiation in the same manner as the previous trials. For this trial, there was one 30-second irradiation session followed by 48 hours of incubation.

CONCLUSION

The poor results were likely due to a lack of resources. Cross-contamination between the control and UV exposed side of the Petri dish is also likely which allowed for increased reproduction in the irradiated side; the control and experimental groups were in the same Petri dish and the partition was not airtight. In the future, more trials would be conducted to provide statistically significant results. Additionally, the control and independent variables would be separated into different dishes to avoid cross-contamination. Larger UV lamps with a wavelength closer to 264 nm would be used because it is likely that higher UV-C wavelengths are more effective.

This research will continue to look into the effectiveness of UV radiation as a germicidal agent. Further experimentation can include using UV radiation against bacterial accumulation on students' cell phones as well as UV radiation on ground beef samples.

METHODS

Tests on the effectiveness of UV radiation in the eradication of *E. coli* consisted of two 3-part trials. For the first trial, three Petri-dishes of *E. coli* were cultivated at the following temperatures: 38˚C (roughly the temperature of human intestines), 24˚C (room temperature), 5˚C (temperature of the average home refrigerator), and 1˚C (temperature of a standard meat cooler). The three dishes were divided in half via a piece of cardstock inserted into the nutrient agar; one side of each dish served as the control while the other side was treated with a 30-second dose of 254 nm UV-C radiation twice a day and then incubated for 24 hours.[6] This process was repeated for five days. UV-C light was administered via a portable UV-C light wand composed of a 3W DC lamp. Since we did not have the tools to measure the pre-treatment colony density and post-treatment colony density, our findings were only visual observations and estimated calculations from measurements of the colonies' radii. Area estimates were done by measuring the four radii from the center to the furthest extent of the colony in four directions. These measurements were averaged, and a circular area was calculated from this number.

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The intent of these trials was to confirm UV radiation's sterilization abilities and explore using UV radiation to destroy bacterial colonies. In the future, we hope to further explore the applications of UV sterilization.

Equipment

This research used 12 sterile Petri dishes, Carolina® Nutrient-rich agar, Caroline® K-12 non-Shiga toxin-producing *E. coli*, and a portable Socean-UV Germicidal UVC 3W DC lamp.

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