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Article

The effects of UV-C and ionizing radiation on the functions of *Escherichia Coli*

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SUMMARY

Planetary protection is an extremely important consideration for spacecraft missions to other planets and worlds, especially those that may harbor life. This work presents the effects of Ultraviolet (UV) C and ionizing radiation on *Escherichia coli* (*E. coli*) bacteria in order to characterize the behavior of bacteria in space to better inform planetary protection considerations. We sent a sample of *E. coli* K-12, a commonly available strain, on a NASA sounding rocket to space via the Cubes In Space™ program, a global competition that allows students to design and propose experiments that launch on a sounding rocket. Our hypothesis was that the ionizing and UV-C radiation experienced during rocket launch and spaceflight will negatively affect the cellular functions of the *E. coli* bacteria and kill some bacterial cells but will not exterminate the entire culture of bacteria. Our experiment demonstrated that the number of colony forming units (CFUs) in the space sample was lower than the control sample. Thus, we suggest that the UV-C and ionizing radiation experienced during rocket launch negatively affects the cellular functions of *E. coli*.

INTRODUCTION

Planetary protection is an extremely important consideration for spacecraft missions to other planets and worlds, especially those that may harbor life, such as the planet Mars, and Jupiter and Saturn's outer moons, Europa and Enceladus (1). Planetary protection is the practice of preventing planets from being contaminated by life from Earth, as well as shielding Earth from potential life forms that could return from other celestial bodies (2). Microorganisms are often unintentionally brought aboard spacecraft, despite standard clean-room and sterilization procedures. When visiting worlds that could harbor life, bringing along microbes from Earth is dangerous for two reasons. First, Earth microbes may be detrimental to any existing native life on the visited world. Second, when looking for signs of life, any detected life might be microorganisms brought with the spacecraft from Earth rather than native life (3). Though space is a harsh environment with high amounts of radiation exposure, some microbes are still able to withstand the environmental pressures. Understanding how microbes react to radiation exposure in space is essential to taking precautionary measures here on Earth to sterilize spacecraft against biological contaminants.

In order to inform about planetary protection requirements, we aimed to understand how the cellular functions of a common strain of bacteria, *E. coli*, are affected by exposure to high doses of radiation that occur during a rocket's launch into space. In this experiment, the change in CFUs is used as a proxy for the change in cellular functions as a result of radiation exposure. The CFU is a unit of measurement used to determine the number of viable cells that are present in a given population of cells. *E. coli* is a coliform (rod-shaped) bacterium that is often found in the large intestine of warm-blooded animals (4). *E. coli* is a type of bacteria that can survive in both the presence and absence of oxygen.

Ionizing radiation consists of subatomic particles and photons that can directly or indirectly eject an electron from an atom (5). Ultraviolet C (UV-C) radiation is the invisible solar rays that travel to the Earth's atmosphere and are absorbed by the Earth's ozone layer (6). These rays are between visible light and x-rays in the electromagnetic spectrum, with a wavelength of 100 to 280 nm (7). Earth-based laboratory experiments have shown that these kinds of radiation are detrimental to cells and organic material. When ionizing radiation comes into contact with cells, it can inhibit the DNA from replicating properly and damage the DNA to the point that the cell dies (8). However, replicating the exact radiation environment experienced during rocket launch and spaceflight on Earth is difficult.

Cubes In Space™ is a global competition in which students aged 11-18 years old design and propose experiments to launch either into space onboard a NASA sounding rocket or to a near-space environment on a zero-pressure scientific balloon. The purpose of this experiment is to learn more about the behavior of bacteria in space to better inform about planetary protection considerations. This experiment requires the bacteria to be exposed to the space environment, which is why the sounding rocket was chosen over the balloon as the launch vehicle. Several research studies have been conducted on the effects of UV radiation on *E. coli* while on Earth. For example, the American Society for Microbiology Journals published a research article on UV inactivation of pathogenic and indicator microorganisms, including *E. coli* (9). There have also been research studies on the effects of *E. coli* in space. NASA previously developed the EcAMSat (*E. coli* AntiMicrobial Satellite) CubeSat mission that tested the effect of micro-gravity on the antibiotic resistance of *E. coli* (10) but did not specifically test for the effect of radiation exposure. The cell growth rates for the two strains of *E. coli* aboard the EcAMSat were unaltered by microgravity (11).

We aimed to test the effect of radiation on the cellular functions of *E. coli* in the space environment though the Cubes In Space™ program. We hypothesized that the

Figure 1: Cubes In Space™ Rocket Launch (12). A view of the rocket launch used for the Cubes In Space™ Program.

ionizing and UV-C radiation experienced during rocket launch and spaceflight will negatively affect the cellular functions of the *E. coli* bacteria and kill some bacterial cells, but will not exterminate the entire culture of bacteria. Results from this experiment will help scientists better develop anti-microbial measures to sterilize spacecraft before launch and characterize how bacteria respond to the space environment.

RESULTS

On Thursday, June 20, 2019, a NASA sounding rocket launched with this experiment onboard from the NASA Wallops Flight Facility in Virginia, as shown in **Figure 1**. We expected to see a decrease in the CFU of the bacterial culture that experienced rocket launch and spaceflight compared to control samples, but we did not expect a complete extermination. The rocket encounters the space environment for 15 minutes during its flight, which is likely not enough time to affect all the bacterial cells.

To measure the effect of space-borne radiation, two vials of *E. coli* bacteria were prepared, identical in composition. One vial remained on Earth and served as the experiment control, while the other vial was launched to space. Two *E. coli* samples were created and employed in total, each on a separate petri dish. One sample was taken from the *E. coli* vial that remained on Earth to serve as the control sample.

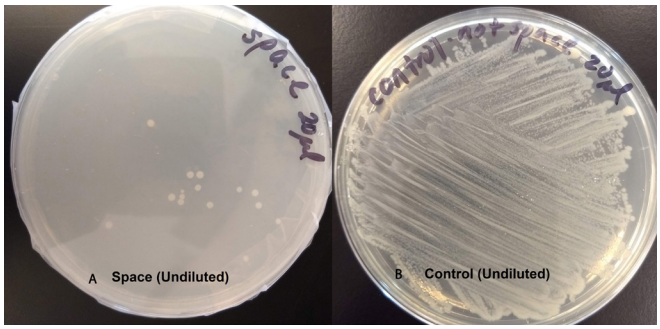

Figure 2: (A) Space Sample Undiluted. 20 µl of an undiluted space-treated bacterial sample was spread evenly across a nutrient agar plate and incubated at 37°C for 72 hours prior to counting. (B) Control Sample Undiluted. 20 µl of an undiluted control bacterial sample was spread evenly across a nutrient agar plate and incubated at 37°C for 72 hours prior to counting.

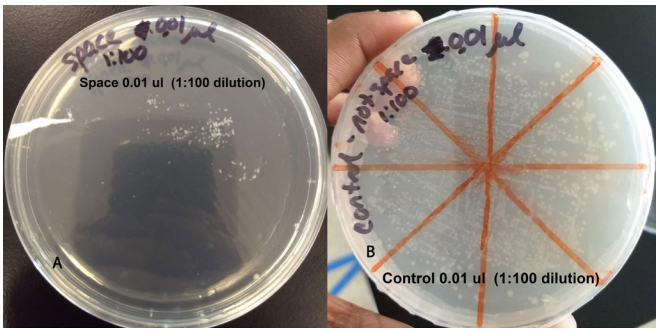

Figure 3: (A) Space Sample Diluted. 20 µl of a space-treated bacterial sample serially diluted 1:100 in nutrient broth was spread evenly across a nutrient agar plate and incubated at 37°C for 72 hours prior to counting. (B) Control Sample Diluted. 20 µl of a control bacterial sample serially diluted 1:100 in nutrient broth was spread evenly across a nutrient agar plate and incubated at 37°C for 72 hours prior to counting.

The other sample was taken from the *E. coli* vial that was sent to space to serve as the experiment (space) sample. The undiluted space sample of the *E. coli* post-flight contained 15 CFUs (**Figure 2A**). As seen in **Figure 2B**, it is difficult to discern individual CFUs in the undiluted control sample. Since there were too many CFUs to count on the petri dish, we conducted a 1:100 dilution on both samples. Without the dilution step, counting the number of CFUs in the control sample would not have been feasible because the density of bacterial culture was too high to discern individual CFUs. The diluted space sample is shown in **Figure 3A**, and the diluted control sample is shown in **Figure 3B**.

The results are summarized in **Table 1**. The space sample showed less CFUs in comparison to the control sample, in undiluted as well as diluted conditions.

DISCUSSION

The purpose of this experiment was to study the effects of UV-C and ionizing radiation on the *E. coli* strain K-12 to provide information for improving planetary protection requirements. We compared the number of CFUs in the control sample and the space sample post-flight. The control sample (undiluted) contained approximately 100,000 CFUs, while the space sample (undiluted) contained 15 CFUs. We conclude that the UV-C and ionizing radiation experienced during rocket launch and spaceflight decreased the number of CFUs in the bacteria. The UV-C and ionizing radiation negatively affected the cellular functions of the *E. coli* bacteria, as predicted.

It is evident from data shown in **Table 1** that the number of CFUs in the *E. coli* bacteria culture exposed to UV-C and ionizing radiation in space was lower than the number of CFUs in the control sample. We demonstrated that the radiation exposure in space could affect *E. coli* survival. However, the radiation did not fully exterminate all the CFUs. Our observation that the exposure to spaceflight did not fully

Table 1: CFUs in Space and Control Samples

	Space Sample (Undiluted)	Control Sample (Undiluted)	Space Sample (Diluted 1:100)	Control Sample (Diluted 1:100)
CFUs	15 CFUs	100,000 CFUs	0.15 CFUs	1,000 CFUs

eliminate all bacteria implies that the radiation exposure during at least the rocket launch may not be sufficient for planetary protection requirements; enhanced sterilization procedures are required. However, the entire decrease in the number of CFUs cannot fully be attributed to radiation alone for the following reasons: the space sample had to be delivered to Cubes In Space™ one month before launch and was shipped in a fashion that could not be climate controlled. Notably, the control sample was not shipped to Cubes In Space™ along with the space sample. However, both samples were packaged at the same time pre-flight and were opened at the same time post-flight. Because of these restrictions placed by the Cubes In Space™ program, the space sample faced ambient conditions (e.g. temperature, pressure) for a long time in transit, which also may have contributed to the decrease in CFUs. Once Cubes In Space™ retrieved the experiment, the return shipment process took two months. In these two months, the space sample again faced prolonged ambient conditions, which could have also led to the decrease of CFUs. However, our results show that even in these prolonged ambient conditions before and after radiation exposure, some bacteria were able to survive. This highlights the importance of establishing effective sterilization methods of spacecraft to ensure planetary protection.

In the future, it would be beneficial to conduct additional studies to better characterize the effects of radiation on bacterial viability. The first suggestion is to conduct multiple trials for counting the number of CFUs in each sample to reduce the effects of unmodeled conditions. The second suggestion is to conduct an experiment on another species of bacteria, such as *Staphylococcus aureus* (a gram-positive bacteria), to understand the different responses to UV-C. The third suggestion is to ship the experiment in a climate-controlled fashion. The fourth suggestion is expedite the transit of the bacterial samples. The fifth suggestion is to include a radiation sensor to measure the accumulated radiation experienced in-flight. The last suggestion is to characterize the extent of DNA damage, if any, in bacterial cells treated with UV-radiation using DNA sequencing.

MATERIALS AND METHODS

Two 4 cm^3 identical plastic cubes that each weighed 11 grams were prepared: one to keep on Earth as the control and one to send to space as the experimental sample. The procedure to prepare each cube was as follows: first, one sample of bacteria was prepared by safely pouring half of the liquid *E. coli* strain K-12 bacteria in a vial. The liquid *E. coli* strain K-12 bacteria was obtained from the Carolina Biological Supply (13). Then, the inside edges of a cube were wrapped with foam to prevent vial damage. Then, a layer of metal washers (weighing a total of 40 grams) was placed at the bottom of the cube in order to meet the weight requirements set by Cubes In Space™. The vial was placed on top of the layer of metal washers. Finally, the cube was sealed with tape. The control sample that stayed on Earth was stored at room temperature in a closet and the space sample was sent to Cubes In Space™.

The following is the launch concept of operations (14). Each bacterial space sample used for this experiment was shipped to the Cubes In Space™ office in Norfolk, Virginia. Once Cubes In Space™ received the experiments, payloads were shipped to the Colorado Space Grant Consortium at the University of Colorado at Boulder. University students placed the 80 payloads into a canister, and the canister was shipped to the NASA Wallops Flight Facility. The canister was then placed and sealed into the rocket. After the rocket had launched and returned to Earth, a boat retrieved the rocket from the ocean and the rocket was disassembled. The bacterial samples were then shipped back to the respective participants.

The steps for analyzing the total viable CFUs (undiluted) were as follows: space sample bacteria were re-suspended in 2.5 mL of nutrient broth (Carolina Biological Supply). A 20 µL aliquot of the space sample was added to nutrient agar plate and 20 µL of the control sample into another nutrient agar plate. A sterilized inoculation loop was used to spread the sample across each petri dish. The bacteria were grown at 37°C in an incubator for 72 hours. Finally, the number of colonies in each petri dish was counted using a dissection microscope or magnifying glass. Similar methods were used to analyze CFU counts in diluted samples, where a 1:100 dilution was performed with additional nutrient broth. A 1:100 dilution is a mixture of 1 part of the solution and 99 parts of additional nutrient broth (15).

For the diluted control sample, the petri dish was divided into eight sections with a marker, as seen in Figure 3B. The equation employed in this analysis to determine the total number of CFUs in the undiluted control sample (Figure 3B) is as follows:

$$Y = abc$$

a is the number of CFUs in one section, *b* is the number of total sections, and *c* is the dilution factor. In this experiment, the variable *a* is determined to be 125. The variables *b* and *c* are parameters, which are chosen to be b = 8 and c = 100. Thus, Y is 100,000 CFUs:

$$Y = abc \quad (1)$$
$$Y = 125 \times 8 \times 100 \quad (2)$$
$$Y = 100{,}000 \ CFUs \quad (3)$$

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