The Effects of Ultraviolet Radiation on Human Skin Normal Flora and Shielding Properties of Minerals

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Abstract

Normal flora is microorganisms located on various parts of the human body (skin and mucous membranes) of healthy individuals, if these bacteria were to mutate the protection they provide to their human host could be jeopardized. At an altitude of ~ 30 km, the atmosphere will be relative to conditions that can be expected on Mars. The intention of this experiment is to send cultures of *Staphylococcus epidermidis* and *Staphylococcus aureus* to an altitude of roughly 30 km to expose them to UV radiation to measure the mutations they acquire. The protective qualities of minerals will also be examined during the experiment. The protective quality of the minerals will be measured by examining the shielded bacteria against the unshielded bacteria using cutting enzymes and gel electrophorese.

1. Introduction

Normal flora are microorganisms located on various parts of the human body (skin and mucous membranes) of healthy individuals [1,2,9]. These bacteria serve an important role in human health by producing compounds and environments that reduce the risk of infection by other pathogenic (disease producing) bacteria [3]. However, changes in the host immune system/function, such as space travel (which has a deleterious effect on the immune system [4-6]), can change normal flora into opportunistic pathogens [7]. To prevent this change, we will investigate the properties of unique materials relative to ultraviolet (UV) light. The specific materials used have distinct abilities to interact with UV light because of their composition color, cleavage, and refractive optical abilities

The ozone acts as a filter, it protects us from UV radiation, wavelengths, $200nm < \lambda < 360nm$, coming from the Sun [29]. However, the presence and density of the ozone is relative to the distance from the surface of the Earth, moreover the ozone is not uniformly dispersed in our atmosphere. The majority of the UV-A and UV-B is blocked by the ozone in the Stratosphere [29]. Consequently, as ozone decreases in density the amount of UV radiation will increase relative to the loss. A lack of ozone allows for more UV radiation, which will allow for a better chance of UV exposure for the satellite. There is a relationship between the amount of UV radiation and the distance from the Sun. Newton described the force of an object in relation to the mass of an object in the equation.

The UV radiation exposure in the stratosphere will be similar to what can be expected on Mars. With relatively small amounts of ozone on Mars more UV radiation will reach the surface than here on Earth. As our satellite will be traveling above Earth's ozone layer it will experience an exposure level closer to the Mars environment. However, as Mars is further away from the Sun the actual exposure on Mars would be less than in Earth's upper stratosphere [29]. This inverse square relationship holds true for many measurements of physical quantities, including radiation. The effect of radiation is inversely proportional to the square of the distance between the object being radiated upon, and the source providing the radiation.

In this experiment, our mission is to send a satellite into Earth's stratosphere (~30 km) to observe the effects of UV radiation on human skin normal flora and measure the protective qualities of various mineral compounds to prevent bacterial mutation due to UV radiation exposure. Our payload shall carry three petri dishes (two test and one control) containing *Staphylococcus aureus* and *Staphylococcus epidermis* and four minerals (biotite, talc, gypsum, and graphite) within a heated compartment. Each test petri dish shall be divided into six sections with each species of human cutaneous bacteria occupying three sections per dish. We choose to culture two *Staphylococcus* species for each plate. Graphite and talc will each cover one third of one of the test dishes to provide shielding, with biotite and gypsum each covering one third of the second test dish. The remaining one third of the dishes shall have direct exposure to UV radiation. Our internal control plate containing both *S. aureus* and *S. epidermis* shall be covered with aluminum foil to provide shielding form UV radiation during the experiment. Upon receiving our payload, we shall culture the colonies of both species among our experimental and control groups (at the CCA Biology Laboratory) to determine if the bacteria survive the near space environment. In addition, we shall perform restriction enzyme digest to establish if mutations occurred and compare this data to the external control DNA and parent (original clonal colony for flight bacteria) digest pattern.

Our hypotheses are:

1) The non-metallic light colored samples will have the best shielding affect due to the fact their refraction index number, color, and configuration

2) *Staphylococcus* species exposed to a Mars like environment would survive but acquire nonlethal mutations

3) The lowest mutation rate among *Staphylococcus* species shielded with talc and/or gypson.

2. Design

Using foam core, we constructed a BalloonSat with the dimensions of 16 cm by 14 cm by 20.5 cm. The single compartment was insulted with polyethylene to protect the bacteria and electrical equipment from the varying temperature outside the BalloonSat. The compartment also contained a heater connected to three 9V batteries to maintain the internal temperature, which was essential to cellular respiration. There were two 95 mm holes cut into the foam core for the placement of the two petri dishes and the shielding material overlay to allow direct access to UV radiation. The internal control was covered with aluminum foil to protect the bacterial species from UV radiation. However, we do not believe that the shielding minerals or the aluminum foil would protect the *Staphylococcus* species from other types of radiation such as gamma radiation and cosmic radiation [28]. The bacteria species would also be exposed to low pressure and oxygen levels. Although *Staphylococcus* species grow best in the presence of oxygen, they are facultative anaerobes [8]. Therefore, should survive in the low oxygen environment. On each of the petri dishes, the bacteria will alter between species (figure 1).

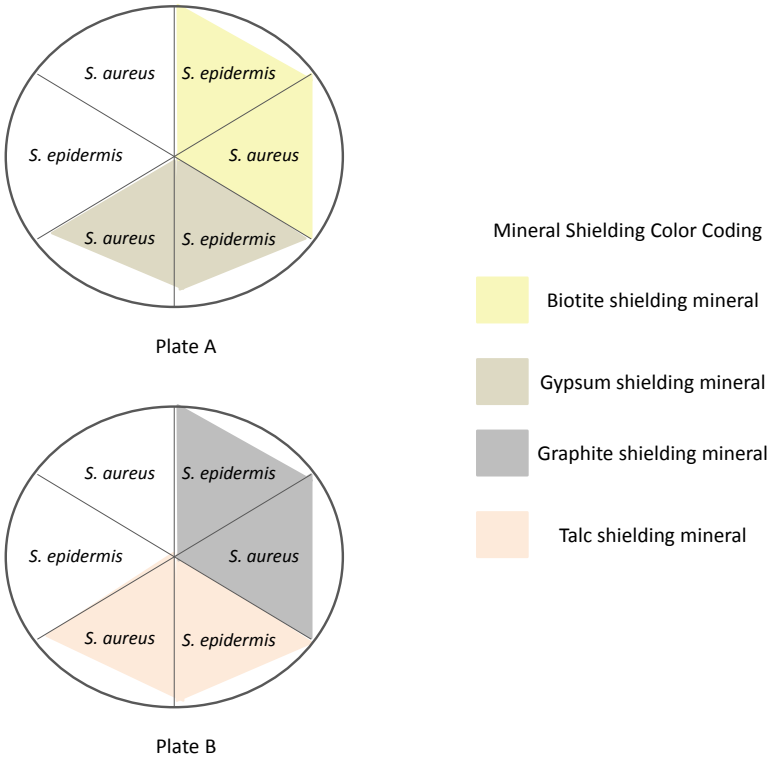

Figure 1. Schematic of bacterial position on the petri dish and the associated shielding mineral overlay.

The Arduino Uno was mounted onto the insulation. The microcontroller board was responsible for recording the temperature, pressure, and acceleration. There is one temperature sensor inside the heated compartment. The accelerometer was contained within the heated compartment while the pressure senor was fixed in a hole of the foam core to measure the pressure outside the BalloonSat. The heated circuit was mounted on foam core powered by three 9V batteries. Two switches located outside the box controlled each the heater circuit and the Arduino Uno. We included a LED light to serve as a visual cue of proper function of the Arduino Uno.

2.1. Testing

Structural Integrity Test

Drop Test

We performed the drop test to simulate landing. This test is one the three tests completed to confirm the structural design of the satellite. The satellite was repeatedly dropped from two stories (6 meters). Mass was attached inside the satellite to simulate the electrical components of the payload. Sterile petri dishes were included in the testing. The satellite experienced minor dents in the foam core; however, no structural modification were needed.

Stair Test

The stair test simulates the landing and drag of the satellite upon return to the ground. The satellite was kicked down the stairs. This test was repeated numerous times using mass simulators for electrical component and sterile petri dishes. No major structural damage was observer following the test. No changes were needed.

Whip Test

The whip test is designed to simulate the volatile conditions the satellite experience following the balloon burst and ensures the payload remain attached to the balloon. The test entailed swinging the satellite erratically by a rope through the tethering apparatus. Again, mass simulators and sterile petri dishes were used during the test. No structural variations were needed following the whip test.

Conditions test

Fight Simulation Test

The cold test was performed in a cooler at -80°C (the sublimation temperature of dry ice) to simulate the extreme cold temperature the BalloonSat may experience during the flight. This test evaluated the temperature control measures implemented in the design of the satellite and functional aspect of electrical components. Therefore, all electrical components needed for the flight were used during this test. The satellite was placed in a cooler with dry ice for three hours. The purpose of the power test was to ensure the power to the Arduino Uno and the heating circuit was sustainable during the flight. The switch to the Arduino Uno and the switch to the heating circuit were turned on. Both units remained on for three hours, the duration of the flight. The power supply selected was sufficient for flight. No additional insulation or heating changes were needed.

Agar Testing

Agar testing was done to ensure the integrity of agar during flight. The petri dish was first placed in a conventional freezer for three hours. We discovered that the liquid phase separate from the gelation in the agar. This information allowed us to set the minimum temperature of 0°C. The next agar test performed was a heating test. A petri dish was placed on a tripod with the heater circuit placed 2.5 cm below the petri dish. This was approximately distance the heater would be away from the petri dish. The heater circuit was turned on for three hours. The integrity of the agar remained uncompromised. No alterations were needed.

3.0 Launch and Recovery

We expect to complete a smooth launched of Project BactoBox at 7:00am (0700h) on 13 April 2013 with heater and Arduino Uno operational via a visual cue of an LED light. The launch will take place at Eaton, Colorado. The BalloonSat will ascended to altitude of 100,000 feet before the balloon burst.

Because we have not yet launch our BalloonSat, we cannot speculate where the satellite will land. If the winds are low, we expect to recover the satellite approximately 30 miles from the launch site. Upon retrieval of the satellite, the petri dishes with the bacteria (sealed with paraffin) shall be placed into a cooler with ice to slow any bacterial growth outside of the experiment. The petri dishes shall be taken back to the CCA biology lab for DNA extraction and culture to detect mutations.

4.0 Results

4.1 Launch

Launch data will be extracted from the SD card from the Arduino Uno upon returning to CCA. We will generate graphs for the internal temperature , and pressure . We anticipate the internal temperature of the BalloonSat will remain within the range of 15 to 40°C. The range is essential for bacterial growth [14].

4.2 Bacteria Data

Immediately upon returning to CCA biology lab, we shall extract DNA from the experimental bacteria and the control bacterial species (external and internal control). Proper handling of specimens will prevent contamination prior to all tests being completed. *S. aureus* and *S. epidermis* cultures will come from the same clonal species. This method ensures that each species in genetically identical and therefore can rule out changes in DNA due to genetic variation among the species. We have designated the petri dish shield with Biotite and Gypsum as plate A and the dish shielded with talc and graphite as plate B.

Selecting a clonal colony will consist of removing a single colony from a particular section from plate A or B with an inoculating loop (figure 5). The criteria for the selection is based on the isolation of that colony from surrounding colonies and the ease of removal. This is illustrated in figure 5. After selecting a colony, we will extract the DNA or inoculate new nutrient agar plates and LB broth with the species to test for survival and mutation. The agar plate will incubate at 37°C for 24 hours for sufficient cell grow and colony development. The LB broth will be allowed to incubate at the same temperature for 48 to 72 hours to obtain adequate bacterial population for further testing. We will follow the selection of the colony again to obtain a genetic identical bacterial species (figure 6).

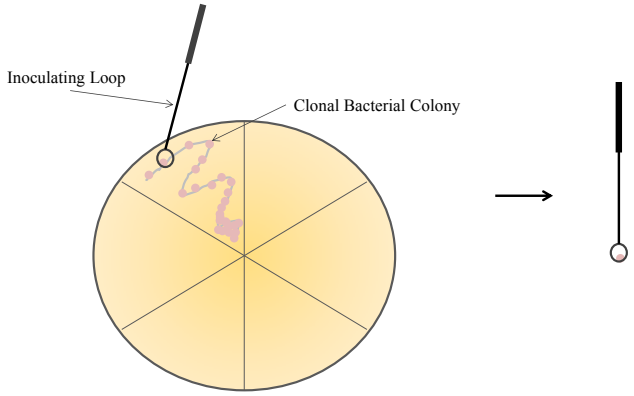

Figure 5. Schematic of colony selection and removal

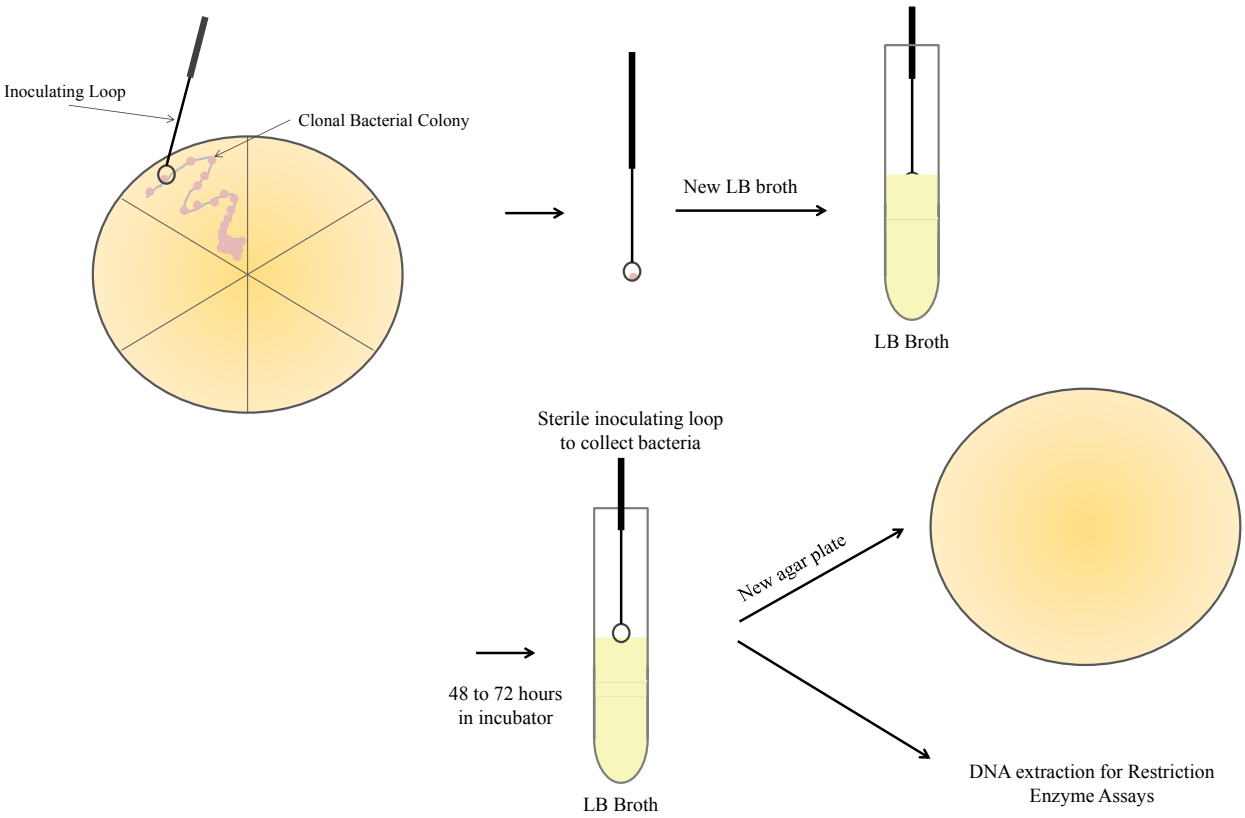

Figure 6. Schematic of isolation and bacterial culture

The protocol for the extraction of the DNA will consist of following manufacture instructions from the Qiagen DNeasy Blood and Tissue Kit. Following extraction, we will quantify the amount of DNA to ensure an adequate amount for further assays .

The method we will used to detect mutations is restriction enzymes followed by gel electrophoresis. The DNA for the assays will be obtained from the LB broth from culture. We will use *Sma*I which cuts the DNA at the 5'CCCGGG3' sequence. We selected *Sma*I because of the amount of published research in the area [15-21]. We will first digest the DNA from each combination of bacteria and shielding mineral with *Sma*I. Following digestion, we will load the DNA in the wells of an agarose gel.

We will run an agarose gel electrophoresis to visualize the DNA product. The process of electrophoresis uses charge to move the DNA from the well towards the bottom of the agarose gel. The DNA is negatively charge. Gel electrophoresis utilize this aspect by establishing a positive charge at the opposite end of the well or bottom of the gel. Therefore, the DNA will migrate towards the positive charge [24]. In this process, we will load the amplicon (DNA amplification product from PCR) and ethidium bromide (added to gel) and a DNA ladder in the wells of the agarose gel [25-27]. After the completion of the gel electrophoresis, we will use UV light to visualize the DNA [24-27].

We expect to observe different bands length. There should be change in the amount of bands. We anticipated this pattern because the restriction enzyme cuts between the pyrimidine (in this case cytosine or C) and purine (guanine or G). The literature suggest that nucleotide in the DNA exposed to UV radiation will pyrimidine dimer (double bond to each other) [12,22,23]. When this occurs the cytosine will not hydrogen bond with guanine its pair to hold DNA in its double helix creating a distortion. This type of mutation may prevent the production of products that may be essential for cellular processes [12].

5.0 Benefits to NASA and Scientific Community

One of NASA's objectives is to "explore the adaptation, survival, and evolution of microbial life beyond Earth" (Objective 6.2) [13]. We designed our experiment to evaluate the effects of a Mars like environment on normal flora and evaluate the protective qualities as various Earth minerals. Destruction of our normal flora has the potential to leave space travelers vulnerable to pathogenic bacteria. Our research will add to the understanding of the effects of radiation exposure on normal flora during space travel.

6.0 Conclusion

We believe the data will support our hypotheses that our bacteria will survive the near space environment, nonmetallic light color minerals will provide the greatest protection against UV radiation among the minerals, and *Staphylococcus* species shielded with Talc and/or Gypsum will have the lowest mutation rate among the shielding minerals.

If the normal flora fails to survive the near space environment, it may have great implication for prolong space travel in areas with high UV radiation. As mention before, previous research has demonstrated that prolong space travel has insidious effects on the immune system which may increase the risk of infection in this environment [4-6, 10]. In addition, pathogenic bacteria exposed to a space environment has an increased potential to spread because of the reduced lag phase which can increase the final cell population of the bacteria [10] and may increase virulence factors in bacterial pathogenic strains [11]. Without the protection of normal flora, especially *S. epidermis* that comprises 90% of human cutaneous normal flora, may leave traveler extremely vulnerable to disease.

The research may be enhanced with the use of pulse phase gel electrophoreses (PFGE). This method is an enhance gel electrophoresis that changes the direction of the current running in the agarose gel which allows the DNA to migrate in a way that produces discrete bands [30]. We did not perform this assay due to accessibility of the PFGE machine. Unfortunately, our DNA sequencer was not working, resulting in us eliminating this assay from our arsenal. The method would have allowed us to detect mutations (nucleotide changes) within the DNA sequence. We hope that in the future we can perform DNA sequencing to detect mutations in our flight bacteria and possibly investigate the chance of back mutations (the correction of a mutation). This could suggest that the normal flora has the DNA correction mechanism to repair UV damage/mutation. This may implicate that normal flora can survive and maintain its original DNA sequence when it is allowed to be in a protected environment away from exposure to UV radiation.

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