Survival of *B. horneckiae* Spores Under Ground-Simulated Space Conditions

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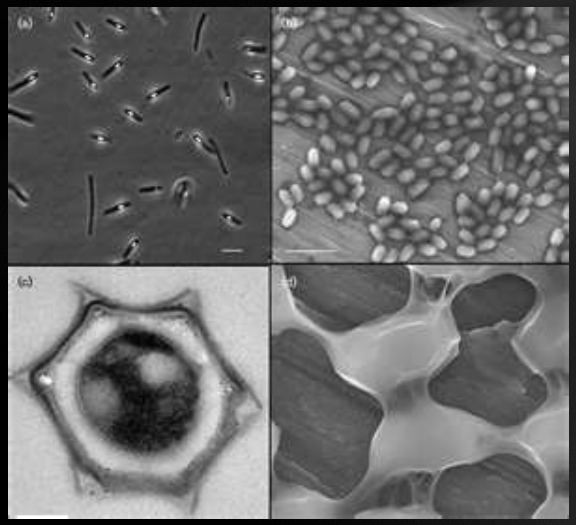
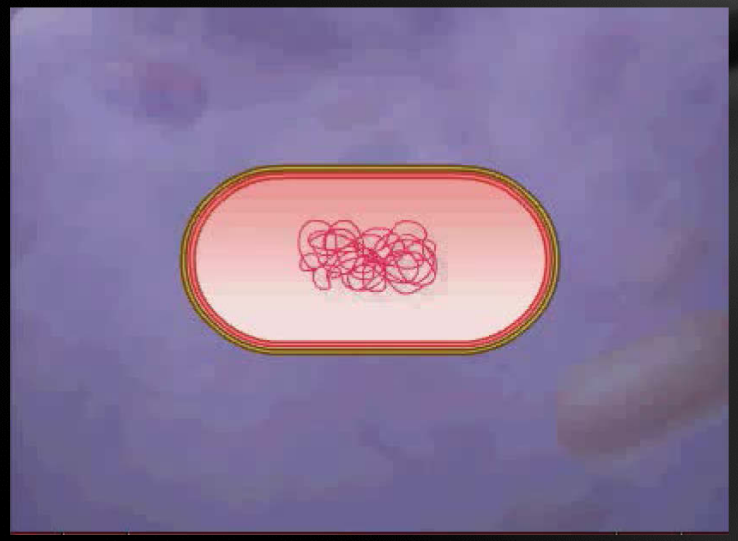
Introduction/Background

- Planetary protection: the prevention of forward and backward contamination during planetary exploration missions
- The International Committee on Space Research established guidelines, enacted by NASA, to ensure spacecraft cleanliness
- Spore-forming bacteria have been isolated from spacecraft and cleanroom surfaces, despite extensive sterilization procedures
  - *Bacillus horneckiae*



Introduction/Background

- Spore-forming bacteria: bacterial strains which form a dormant polysaccharide and protein-coated spore in the absence of necessary nutrients.
  - A means for genetic preservation
  - Spore-forming bacteria have demonstrated unusually high resistance to UV radiation, heat, and chemical sterilization
    - Could potentially withstand harsh extraterrestrial environments



Introduction/Background

- European Space Agency (ESA)'s EXPOSE Missions
  - EXPOSE-E: PROTECT experiment (previously done)
    - Samples of *B. pumilus* SAFR-032 spores were sent to *in situ* (in real) space
      - Carried on board the EXPOSE-E facility, part of the European Technology Exposure Facility (EuTEF) that was attached to the European Columbus Module of the ISS during a February 2008 SpaceWalk
      - After **18 months** of exposure to various space UV conditions, returned to Earth during Discovery's STS-128 mission.
      - Exposure conditions:
        1. Space UV
        2. Dark Space UV (covered exposure)

        } Journey/flight

        3. Martian UV
        4. Dark Martian UV

        } Landing/stay

Introduction/Background

- My project's focus and purpose:
  - **EXPOSE R2: BOSS (Biofilm Organisms Surfing Space) Experiment—Dr. Petra Rettberg (PI)**
    - Analyze samples of *B. horneckiae* spores that underwent 12-month exposure to (ground simulated) various UV conditions: space UV; dark space UV; Mars UV; dark Mars UV
    - Analyze the survivability rates of *B. horneckiae* spores with and without spore coats under all four UV conditions
    - Ultimately, samples of *B. horneckiae* spores will be sent to real (*in* situ) space at the end of 2012

Hypotheses

- *B. horneckiae* spores would show a higher survivability rate under Dark Space UV conditions than those exposed to direct Space UV; similarly, spores under Dark Mars UV conditions would show greater survivability than those under direct Mars UV radiation
- Space UV would be more detrimental to spore survivability than Martian UV radiation
- Spores without exosporium coat would have a significantly lower survival rate than those with the exosporium coat
- Overall, spore survival rates would be significantly lower under all conditions

Methodology

1. Spores underwent ground simulation of UV and Martian conditions in Germany

Methodology

Spore viability assessment

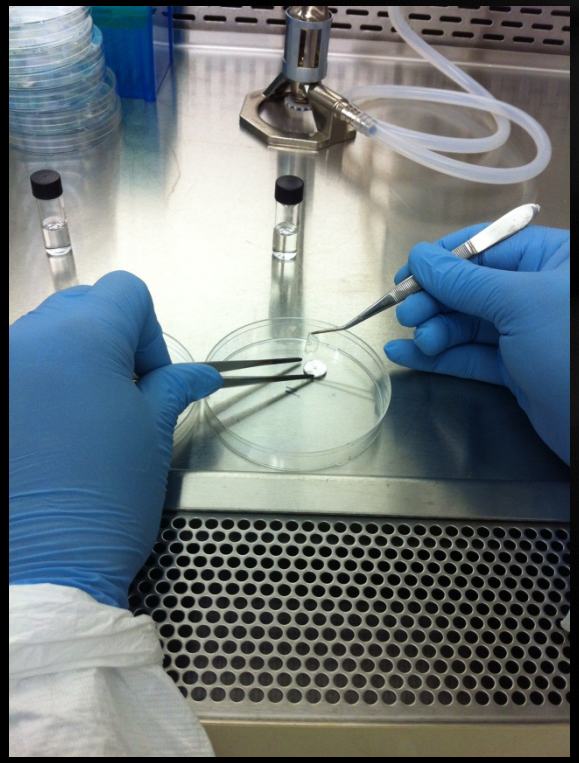
2. Each coupon was covered with approximately 60µL of 10% Polyvinyl Alcohol (PVA), and was incubated at 40-45° C for ~60 minutes, until dry

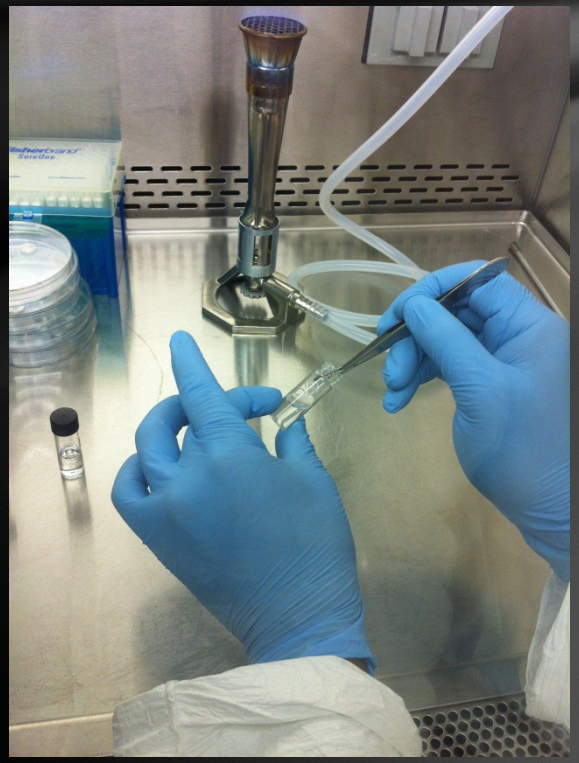
Methodology

3. 2mL of sterile deionized water was pipetted into 5mL vials for each coupon

4. After the PVA films dried, they were removed with forceps and tweezers and deposited into the 2mL of water and vortexed.

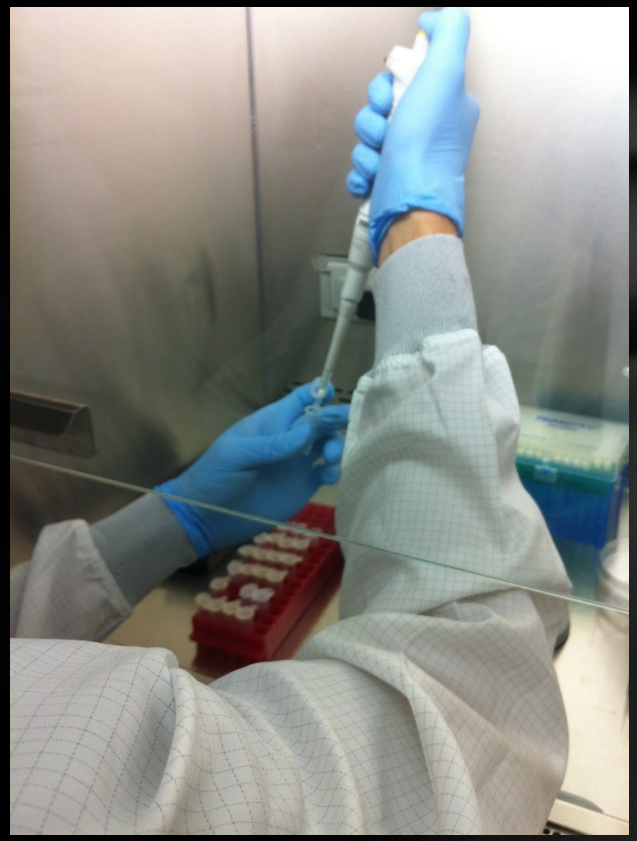
*These steps were repeated twice





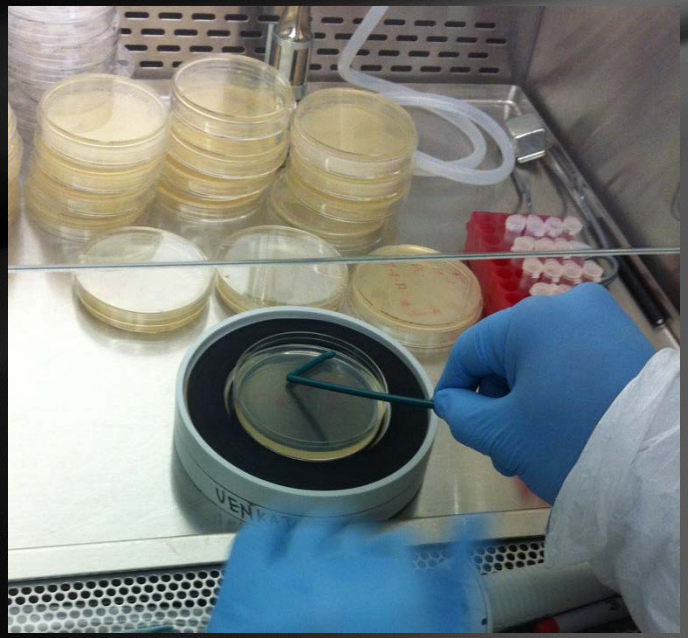
Methodology

4. Serial dilutions to 10^{-4} concentration for each coupon were prepared



Methodology

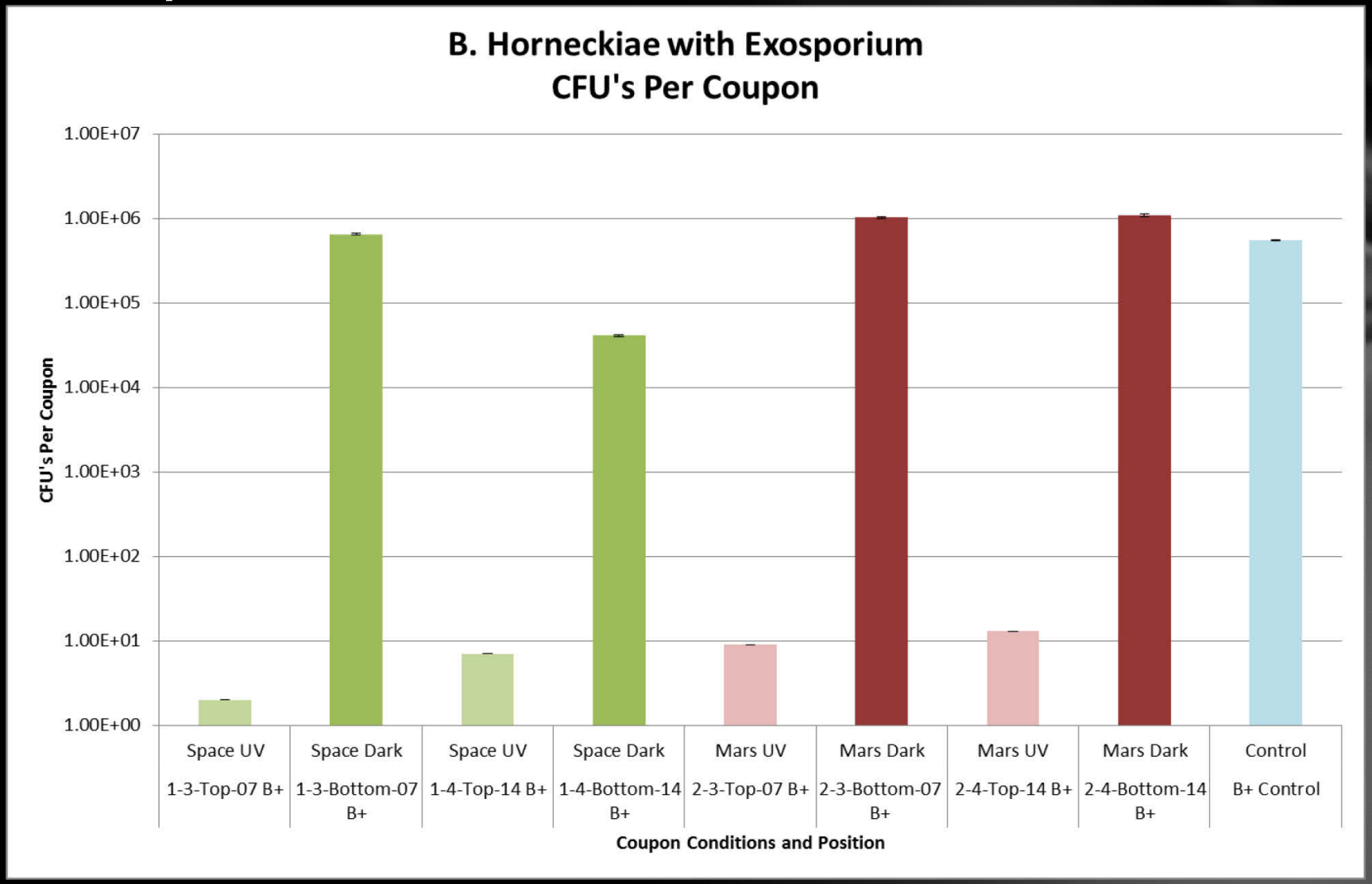
5. 100µL of each dilution was spread plated.
6. Each plate was incubated at 32˚C for 24 hours and 48 hours.
7. Colony counts for each plate were taken after each time interval.



Data/Results

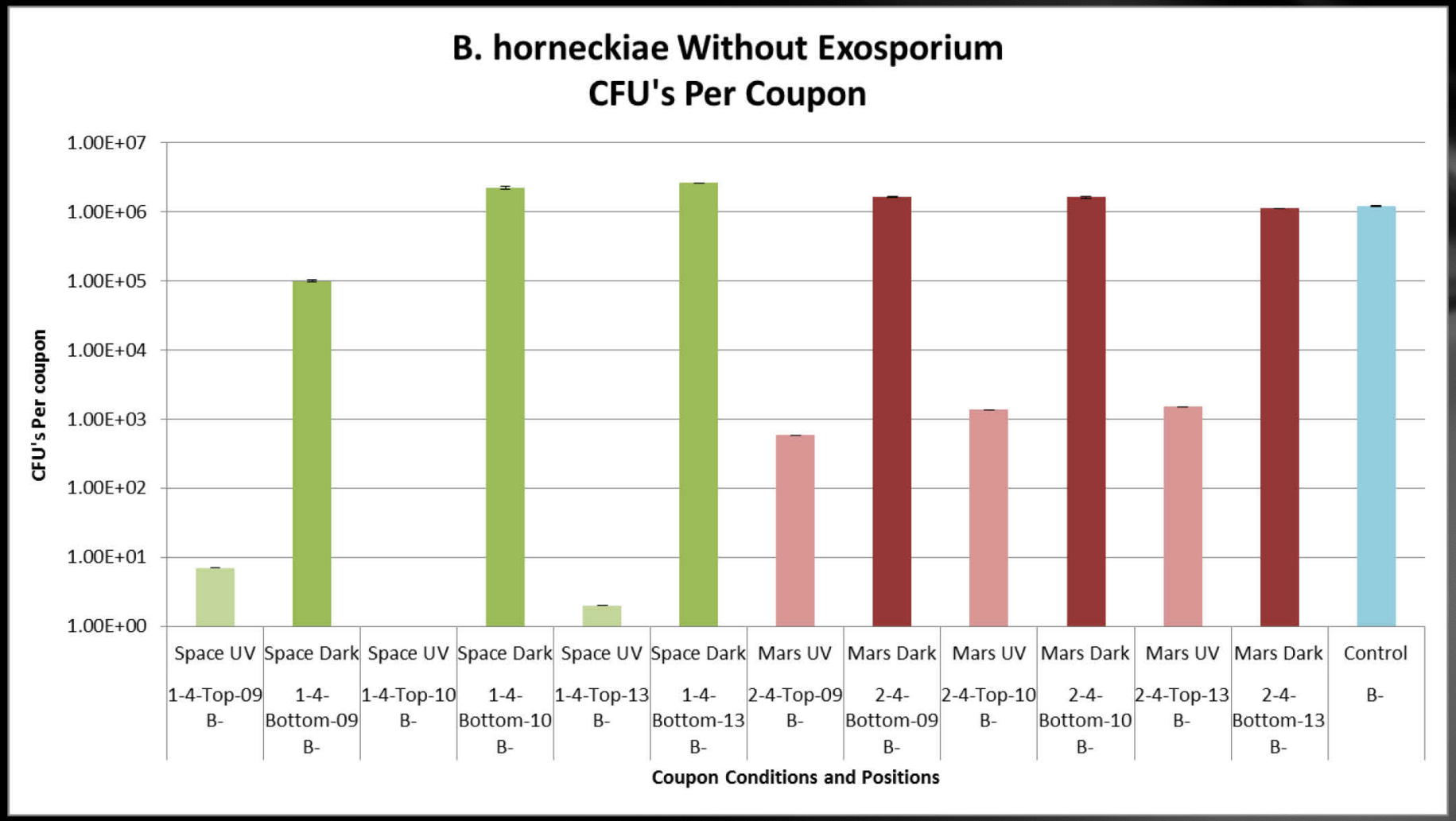
Sample Name	Conditions	CFU's per coupon
1-3-T-07 B+	Space UV	2.00E+00
1-3-B-07 B+	Space Dark	6.56E+05
1-4-T-09 B-	Space UV	7.00E+00
1-4-B-09 B-	Space Dark	9.90E+04
1-4-T-10 B-	Space UV	1.00E+00
1-4-B-10 B-	Space Dark	2.23E+06
1-4-T-13 B-	Space UV	2.00E+00
1-4-B-13 B-	Space Dark	2.58E+06
1-4-T-14 B+	Space UV	7.00E+00
1-4-B-14 B+	Space Dark	4.12E+04
2-3-T-07 B+	Mars UV	9.00E+00
2-3-B-07 B+	Mars Dark	1.03E+06
2-4-T-09 B-	Mars UV	5.79E+02
2-4-B-09 B-	Mars Dark	1.64E+06
2-4-T-10 B-	Mars UV	1.36E+03
2-4-B-10 B-	Mars Dark	1.61E+06
2-4-T-13 B-	Mars UV	1.49E+03
2-4-B-13 B-	Mars Dark	1.12E+06
2-4-T-14 B+	Mars UV	1.30E+01
2-4-B-14 B+	Mars Dark	1.10E+06
B+ Control	Control (B+)	5.58E+05
B- Control	Control (B-)	1.19E+06

Data/Results



*1:Space Uv; *2: Mars UV; *T: Top layer; *B: Bottom layer; *B+: With Exosporium; *B-: Without Exosporium

Data/Results



*1:Space Uv; *2: Mars UV; *T: Top layer; *B: Bottom layer; *B+: With Exosporium; *B-: Without Exosporium

Conclusions

- For both Space and Mars UV top layers, spores showed a significantly lower survival rate than under dark (bottom layer) UV conditions
- For the most part, spores showed greater survivability under Mars UV than under Space UV conditions
- While UV radiation does negatively affect spore survivability, *B. horneckiae* spores do have the potential for survival under UV radiation, especially under dark/shielded UV radiation.
  - Suggests the presence of UV-resistant mechanisms
  - Spores could potentially withstand the harsh environments of extraterrestrial bodies/planets.

Experimental Concerns

- Variability in the performance of each step for each sample
- Possibilities of sample contamination
- Serial dilution errors
- Time constraints
- Human/manual error

❖ These issues were minimized by: performing the experiment in triplicate; averaging data during analysis; improving time management; and taking great care and attention to minimize errors and contamination during each step of the experiment.

Learning Experiences

- Lab procedures/protocols
  - Basic microbiology techniques: nutrient media preparation; various microbiological culturing techniques (streak, spread, stabs); pipetting; dilution series; spore purification, and spore inactivation
  - Molecular techniques: DNA extraction; PCR and qPCR.
  - microscopy; long duration archiving of microbial strains.
- Statistical analysis
- Designing, planning and execution of lab experiments
- Effective communication of research ideas and results.
- Project report writing

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