Monitoring the High Altitude Survivability and Mutations of Bacillus Subtilis

Demosat Launch Final Report

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NASA Space Grant Program
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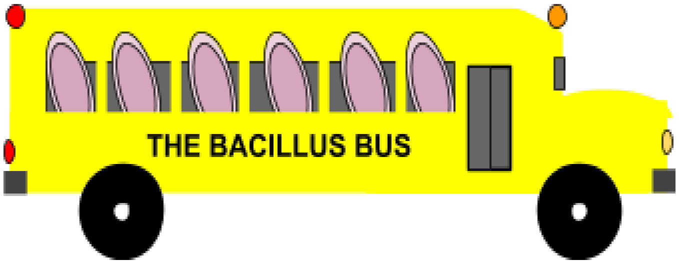

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Abstract

In recent years, the desire for humanity to explore the outer reaches of our solar system has grown substantially. Mars, being the primary source of interest, has shown strong potential in providing a reasonable future for colonization. Agriculture is vital to the success of colonial efforts; thus, the goal of this project is to show the viability of Bacillus subtilis as an agricultural catalyst through its survival in near space environments. B.subtilis is a natural soil inoculant and fungicide. These properties paired with B.subtilis' ability to protect itself from environmental hazards through a process called sporulation, make it of particular interest for further investigation. To determine if this bacteria is viable for space travel, two groups of this strain of bacteria (sporulated and unsporulated) were sent up to ~100,000 ft of altitude in a carefully designed container monitoring the atmospheric conditions at this altitude, such as: temperature, pressure, and radiation. These readings were recorded to an SD card (also located in the container) throughout the duration of the flight. Additionally, genetic mutations were tested after the experiment was concluded to determine what mutations (during flight) may have aided in the survivability of B.subtilis. Upon retrieval of the samples, phenol red and agar tests concluded that the sporulated bacteria colonies retained a higher survivability rate compared to the unsporulated colonies; and that from further testing, no genetic mutations occurred during the test period. From analyzing the recorded data, atmospheric conditions indicate that ‘near Mars conditions’ were met during flight; proving the validity of the test. Therefore, data would indicate that sporulated subtilis would prove as a positive agricultural candidate for space travel should interplanetary colonization occur.

Introduction

Experimental Motivations

The main focus of this report is to test the survivability of Bacillus subtilis when exposed to high altitudes in the atmosphere. Subtilis possess the ability to sporulate itself to protect its genetic properties from harsh environments such as extreme cold, heat, oxygen deprivation, or nutrient deficiency. Therefore, the study outlined in this report focuses on the variation in survivability between a sporulated sample and an unsporulated sample of B. subtilis. Bacteria have fast reproduction intervals allowing more information to be gathered regarding the effects of the environment on the samples. This includes information on genetic mutations. Mutations incurred are apparent in their color response to MacConkey and urea agar plates as well as methyl red (MR) tests.

Standard Bacillus subtilis does not ferment urease or glucose; however, it does ferment lactose. Therefore, unaltered B. subtilis samples will produce a pink stain in MacConkey agar (lactose test), and clear/yellow stains for the urease agar and MR tests (glucose). This pink stain indicates a positive result from the bacteria fermenting lactose whilst the clear/yellow stains in urease agar plates and the MR test indicate a null result for the presence of glucose. Mutations beyond those measurable by MacConkey agar, urease agar, or MR tests can be monitored through visual observation of the bacteria’s growth pattern, density, color, and shape. Variations in the behavior in any one of these parameters indicate a mutation. To test for these mutations, this report makes use of the resources provided by the Colorado

State University's NASA Space Grant to construct a payload within the parameters layed out within the summer 2023 DemoSat program.

Payload Requirements

In order to confidently go into launch with minimal equipment risk, a well rounded testing was performed and operation procedure was established. Testing included ensuring the heaters work as intended by the software and a sequence of sensors calibration for all of the on board sensors (optimizing to anticipated weather conditions). To care for the bacteria and ensure minimal contamination, the payload was sanitized before the addition of the bacterium to their housing and structural stability was checking by ensuring all straps and housings are fastened, and fit to prevent slipping. Careful consideration of construction material was taken into account when designing the main payload body to reduce weight and size. The frame of the payload was printed out of acrylonitrile butadiene styrene (ABS) using Prusa MK3 printers and an exterior shell was formed from reflective insulation foam provided by the Powerhouse Energy Campus at CSU.

Additionally, as per the Colorado Space Grant Consortium (COSGC) requirements, the payload could not exceed 1.5kg, must have the center of mass as close to the center of the payload as possible, and must not exceed a $1000 spending budget. Through many design iterations, a final payload was designed and tested for flight day as layed out in this report.

Methods and Materials

Payload Final Design

The payload was designed to be printed on a single printer bed which allows a more rigid structure. This was surrounded by insulation foam on all six sides of the payload with windows placed on adjacent walls. A top frame was designed to add additional support to the payload during ascent and descent, further keeping the internal contents secure. A more detailed and dimensioned drawing of the final iteration can be found in Figure 1.

Additionally, control boxes were designed to house all the sensors with direct contact to heating pads to allow optimal operation in cold of temperatures. Control box 1 was designed to house components deemed to have the highest priority for the mission: the Arduino Mega, geiger counter and lithium ion polymer (LiPo) battery. Control box 2 housed an additional temperature sensor, an altimeter, a humidity sensor and the SD card reader/writer. Full diagrams of these components can be found in Appendix D.

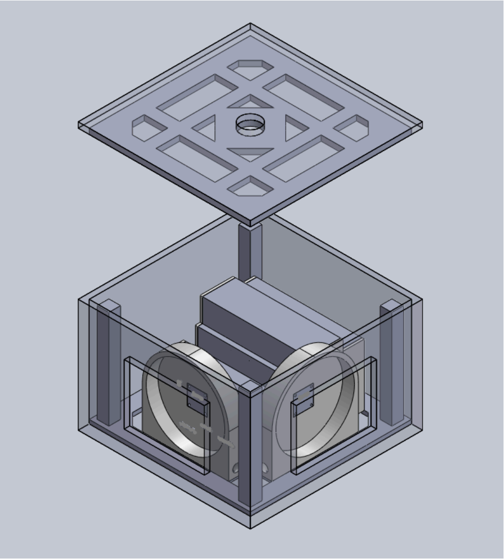


Figure 1: Concept model for Payload V2

Project Management

Due to the limited timeframe of the study (10 weeks), a tentative schedule was developed to allow a reasonable timeframe to produce the payload and experiment found within without running past deadlines (Appendix A). This schedule shows timeframes for some of the more important design requirements to allow testing to begin and conclude prior to the launch. This project met nearly all deadlines on time or ahead of schedule. The freeze test put the largest hole in the schedule as sourcing dry ice proved to be more difficult than anticipated. Additionally, the sourcing of parts was an additional hiccup as the agar petri dishes necessary for cell culturing were delayed.

Final Spending and Weight

The payload specifically was designated a total spending budget of $1000 for parts, materials and post flight testing. Any additional materials used acquired through other means -such as on hand materials- were included in the total budget however not the spending budget. These types of materials would include the insulation foam, initial bracketing screws, and adhesives. Also as per regulations of the competition, this flight allowed each payload a maximum weight of 1.5 kg to be cleared for launch. Before flight day a total of $863.89 was spent and the maximum weight the payload reached was recorded at 1.443 kg meeting both of the requirements. A more detailed parts list with prices and weights can be found in Appendix B.

Test Plans

Preliminary flight test plans were centered around exposing the payload to specific weather and physical conditions similar to what may be experienced during the flight. To elaborate, at 110,000 feet potential temperatures can reach as low as -80 ˚C in some cases and experience severe wind whipping forces capable of payload damage. Additionally, after the payload returns to the ground, it can reach crash landing speeds up to 15-30 mph which poses possible contamination and damage risks.

Proper flight test precautions were therefore developed to design a capable structure to withstand these elemental forces battering the capsule during ascent and descent. Additionally, some sensor calibration was needed to ensure proper data is recorded on the flight.

Test 1: Freezer Test and Results

The freeze test utilized the extreme cold temperature of dry ice to simulate drastically low temperatures at high altitudes along the payload journey. The test is designed to determine if the heaters and insulation inside the payload will be adequate to prevent electronic failures in the instance of extreme cold. A cooler full of 8 lbs of dry ice was used as the simulated environment for this test (Figure 9). The payload was subject to this environment for a maximum of 3 hours among trials (anticipated fly time).

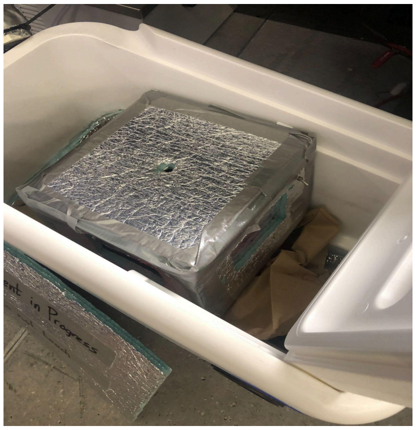


Figure 2: Payload inside of dry ice cooler

This test was performed 3 times, providing difficulty and insight through each iteration. Initially, the cooler had not reaching the required temperature to perform an accurate test. One pound of dry ice was used for the initial study with the assumption the cooler had sufficient insulation to allow the temperature to drop to what we anticipated in the upper atmosphere. This was not the case. The goal temperature was around -50 °C but, with the initial amount of dry ice used, the interior temperature only reached about -10 °C. Following this failed attempt, more dry ice (8lbs) was added to maximize cold exposure. The desired temperature was achieved on the second trial, however, in trial 2 the payload was not properly insulated or sealed resulting in our electronics freezing, causing the Lipo Battery to be put out of commission due to voltage failure. Fortunately, a second identical battery was on hand for future testing and no other electronics were damaged. Finally for the third cold test, the payload was properly sealed, and 8 lbs of dry ice was added to the cooler. After 3 hours, the payload was still operational and data collection was a success.

Test 2: Whipping Test

The whip test involves swinging the payload above one's head via a string to account for any potential dislodging equipment or loose wires. This test is achieved by rapid spinning, severe whipping, and jostling of the payload to simulate turbulence.

Figure 3: Nick performing overhead whip test

Results concluded that the payload was able to withstand ‘hammer swing’ velocities that exceed an angular velocity of 1 revolutions/second with a one meter long rope. Additionally, the payload was able to survive numerous whip motions as well. The only limiting factor was that the string used would break before the payload, however, testing consisted of several ‘whips’ and ‘hammer swings’ before the rope broke from the tensile forces applied. As a result, testing was deemed successful due to successful data acquisition inside the payload as well as no internal compartments being damaged or dislodged.

Test 3: Impact Test

The impact test involves dropping the payload fully assembled with empty agar dishes from a predetermined height to achieve impact velocity similar to launch day. Simulated by dropping the payload off a building to reach an impact speed of at least 20 MPH. If the payload can survive this impact, then it should be able to survive any other expected speed on launch day.

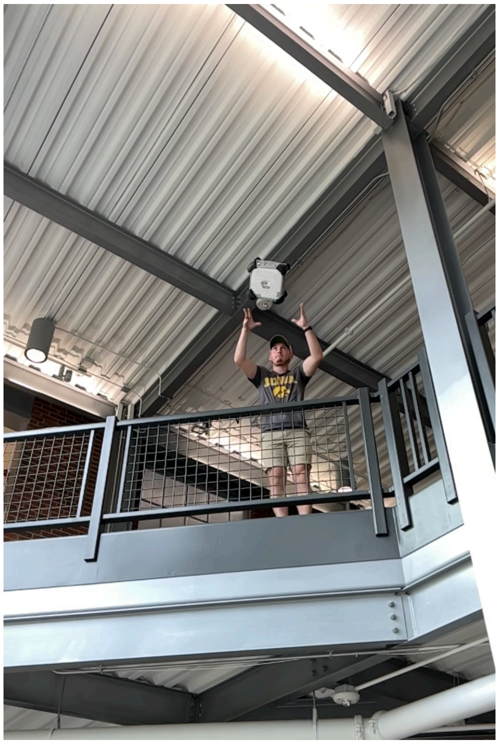


Figure 4: Drop test performed at around 18 ft

Testing was performed based on the drop test manual provided by the DemoSat program. Based on the instructions, the guide recommended that the payload is to be dropped from a range of 15-20 feet onto a hard surface. Therefore, after conducting multiple iterations of dropping the payload off of a ledge at a height of 18 ft, the payload bounced before coming to rest, remaining completely intact with no damage inside or out. Therefore, testing was deemed a success.

Test 4: Vacuum Test

The capacitors inside the geiger counter have the potential to be affected by pressure change, causing failure. If the geiger counter malfunctions, it could also result in a possible short for the rest of the electronics running from the Arduino mega. In order to test how well the electronics in the payload would hold against a sudden change in pressure, a vacuum chamber was utilized provided by the CSU powerhouse.

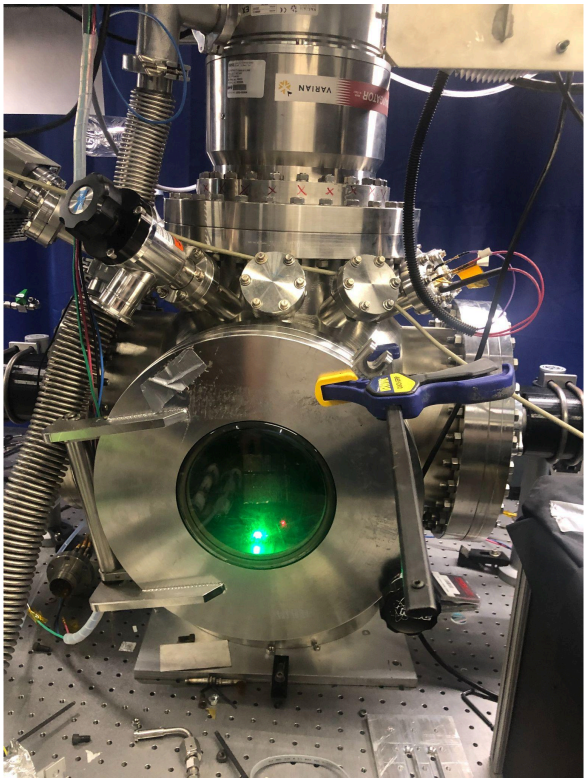


Figure 5: Payload inside of vacuum chamber.

Results of the vacuum test were not as positive as desired. While the data collection continued as normal, the Geiger counter experienced a failure in its high voltage generation. It is believed that the sudden drop in pressure caused a 1N914 diode on board to lose its polarity and allowed back current to destroy the onboard 555 timing chip. This was resolved by replacing the 1N914 with a 1N4937 as the existing one had survived the testing unscathed.

Sensor Testing: Temperature

In addition to the kinetic testing outlined above, ensuring proper calibration of the sensors was deem crucial . The accuracy of the internal sensors will determine not only the survivability of the payload, but will also provide sufficient results relating to the subtilis colonies for post data analysis.

To reiterate, the extreme cold the payload will be subjected to will play a substantial role on the electronics as outlined in the freeze test. Therefore, the temperature sensors must be working optimally to ensure the proper heater operation to minimize battery depletion. Additionally, proper monitoring of conditions the B. subtilis cultures are subjected to is crucial to proper analysis of the survivability.

One of the temperature sensors was placed in an incubator at a set temperature to verify sensor accuratacy. The incubator was set at a temperature of 85.4 °F (26.67 °C) and the respective temperature sensor read a value of 26.61 °C yielding an error of around 0.2% which is within the error parameters provided by the manufacturer. Considering all other temperature sensors showed similar results yielded values varying by 0.01-0.03 degrees in the same environment, it was assumed that all other temperature sensors were properly calibrated.

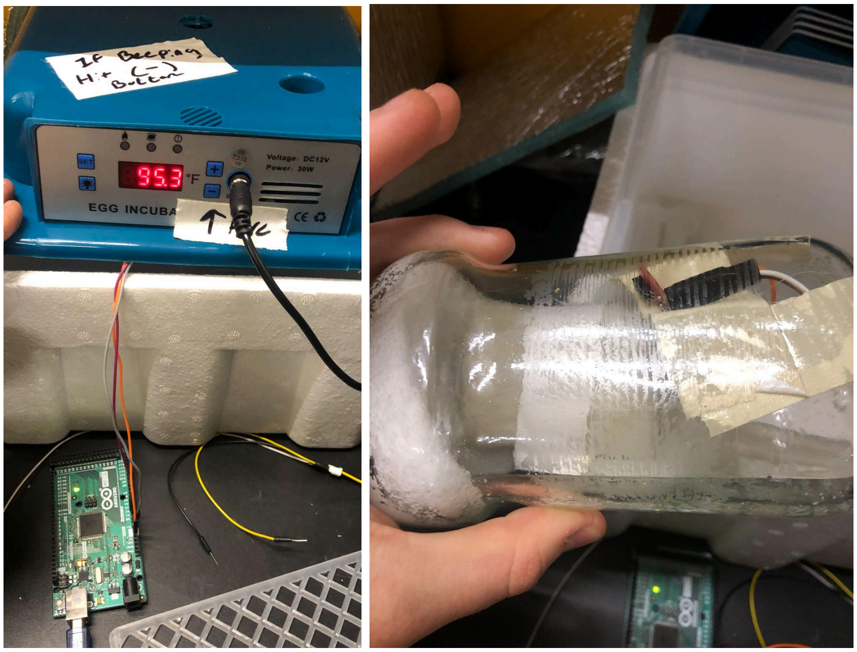
Sensor Testing: Humidity

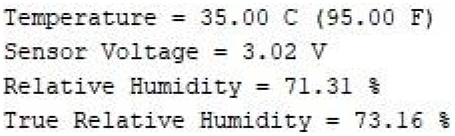
In order to properly calibrate the humidity sensor, special testing was utilized. The humidity sensor was calibrated utilizing a salt test. Essentially, the salt test involves putting the humidity sensor into a jar with a handful of *wet* salt, and controlling the temperature. After a few hours for equilibrium to be established, the salt jar will have a known humidity based on figure 6 (depending on salt type).

Salt	15°C	20°C	25°C	30°C	35°C
Lithium Bromide	6.86	6.61	6.37	6.16	5.97
Lithium Chloride	11.3	11.31	11.3	11.28	11.25
Potassium Acetate	23.4	23.11	22.51	21.61	–
Magnesium Chloride	33.3	33.07	32.78	32.44	32.05
Potassium Carbonate	43.15	43.16	43.16	43.17	–
Magnesium Nitrate	55.87	54.38	52.89	51.4	49.91
Potassium Iodide	70.98	69.9	68.86	67.89	66.96
Sodium Chloride	75.61	75.47	75.29	75.09	74.87
Ammonium Sulfate	81.7	81.34	80.99	80.63	80.27
Potassium Chloride	85.92	85.11	84.34	83.62	82.95
Potassium Nitrate	95.41	94.62	93.58	92.31	90.79

Figure 6: Known humidity concentrations at different temperatures for different salts

Sodium chloride was used to test our humidity sensor. Salt was wetted inside a mason jar, and placed inside an incubator set to 35 °C for four hours to equalize.





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Temperature = 35.00 C (95.00 F)
Sensor Voltage = 3.02 V
Relative Humidity = 71.31 %
True Relative Humidity = 73.16 %
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Figure 7: Humidity Sensor Testing and Recorded Data

Expected humidity data was supposed to read 74.87% as per the chart, our data showed 73.16% which is just over 1% off from the expected data therefore, resulting in an error of 2.3% which was deemed satisfactory for our experiment.

Sensor Testing: Geiger Counter

Another crucial sensor to our study was the geiger counter which would track radiation phenomena during the flight duration. Initially, the readings from the geiger counter seemed troubling as it was recording CPM (counts per minute) in the ranges of 0 - 85 CPM. This is not a typical background radiation reading on the ground under normal conditions. However, with a bit of research it was determined that the average background radiation for the testing location varies between 20 - 60 CPM on average. To ensure proper operation, the geiger was tested using known sources of radiation. Sources of sealed polonium and krypton were utilized to test the functionality of the counter. The payload was sealed, powered on, and placed near the sources. The graph below shows the results of the geiger counter in CPM.

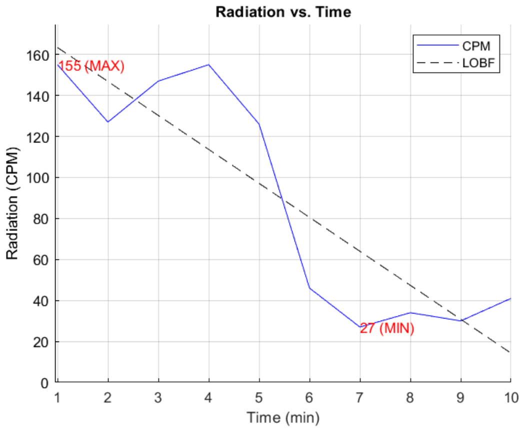

Figure 8: Geiger Counter Test Readings

The test showed the initial placement of the payload near the radiation sources and then the removal of the payload from the sources. Based on the high readings near the sources and the moderate readings away from the sources it was shown that the geiger counter worked as expected and shows the relative randomness of background radiation. This test proved the counter was working properly and there was no need to calibrate or replace the device in the payload.

Launch Day Information

Launch occurred on 07/29/2023 in Limon, Colorado. Before the launch occurred, the payload was inspected one more time for a sanity check, and data was collected briefly just to ensure the SD was not corrupted or malfunctioning. The Bacteria was sanitized and placed into their respective compartments for launch, showing no signs of contamination. All 3 teams were required to show up at 6 am to set up for launch. The expected weather conditions appeared sufficient for launch and would not result in any issues. Photos of the launch can be found in the figures below.

Figure 9: Photos from Limon CO, during launch day.

The flight time lasted 3 hours, and the payloads started their descent by 9am. Upon landing, the payload visibly looked intact, and the agar plates were not cracked. The added indicator LEDs were providing useful error insight upon landing. It was visibly apparent that there was some sort of malfunction that occurred during data collection while writing to the SD. The blue LED meant to indicate data being recorded by flashing once per data collection, was stuck on. This indicated that a malfunction had caused our coded data loop to never reach the end of the loop. Analysis of the data onboard showed data collection occurred normally until about 53 minutes into the mission at a height of roughly 17km. This corresponds with the coldest portion of the assent and the portion with the lowest air density. It is theorized that an electrical failure occurred due to overheating of the electronics as the low air density lacked the capacity to properly convect away enough heat. An interesting note regarding this failure is that whatever occurred was only temporary. Upon retrieval of the payload, additional testing showed that the damage was not permanent. Simply turning the payload off and on again corrected the issue and no permanent electrical damage was found.

Results & Analysis

Bacteria Samples

The bacteria samples and their control counterparts were swabbed and applied to the phenol red, urea, MacConkey, and nutrient agar plates to test for survivability as well as any sort of genetic mutation from their control predecessors.

To reiterate, the survivability of the bacteria was the main focus of this study, as the impacts of sporulated vs unsporulated will play a crucial role in how each colony can withstand such extreme environmental impacts as discussed previously. It was the hypothesis that the sporulated bacteria will withstand such environments with more success comparatively, therefore, to test each sample's survival trait, the sporulated and unsporulated samples were swabbed in 20 different locations and applied to a new agar plate each dedicated to a sporulated group and unsporulated group for visual comparison (Figure 10).

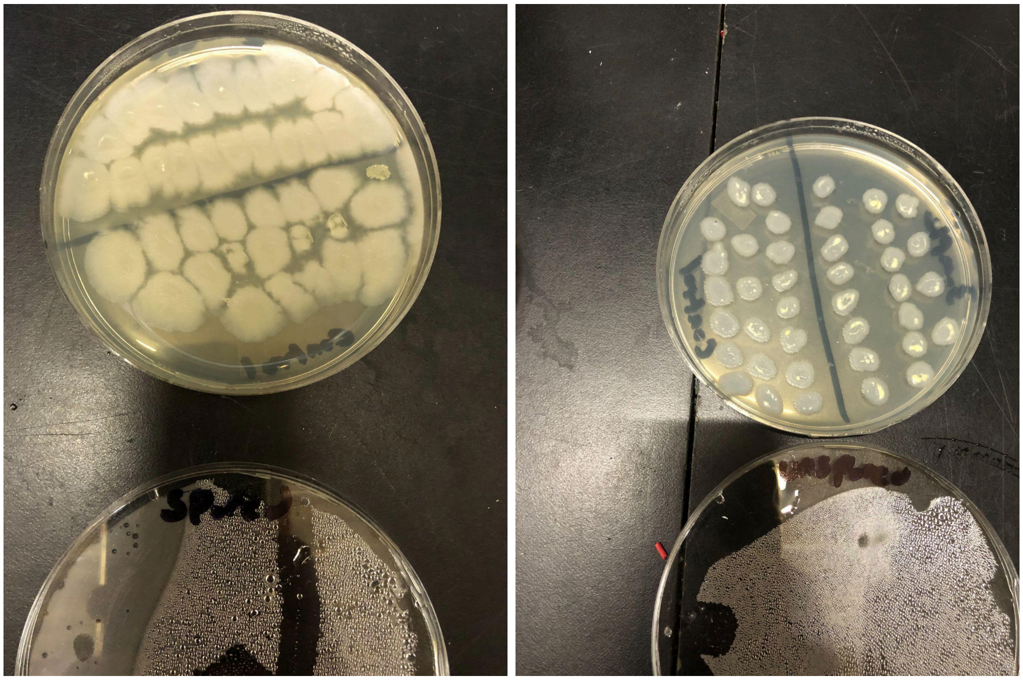

Figure 10: Nutrient Agar plates containing the Sporulated sample and its respective control (left) vs the Unsporulated sample and its respective control (right). Each nutrient agar plate shows the bacteria colony density after launch day.

After 2 days of incubation to allow bacteria regrowth, the sporulated bacteria appeared to have a significantly denser population per sub colony compared to the unsporulated sample. Furthermore, the visual data indicates that while the unsporulated bacteria technically ‘survived’, due to the reduced visual density of each sub colony, there were significantly fewer survivors as compared to the sporulated colonies. Therefore, we fail to reject the hypothesis that sporulated bacteria are able to better survive at altitude.

Regarding the idea of any genetic mutations that could potentially have occurred over the course of this study to consequently aid or harm the probability of survival of Bacillus subtilis; there were no mutations. After each control and sample set of bacteria were swabbed from their agar plates and applied to the phenol red, urea, and MacConkey agar, there appeared to be no visible difference between the samples and controls.

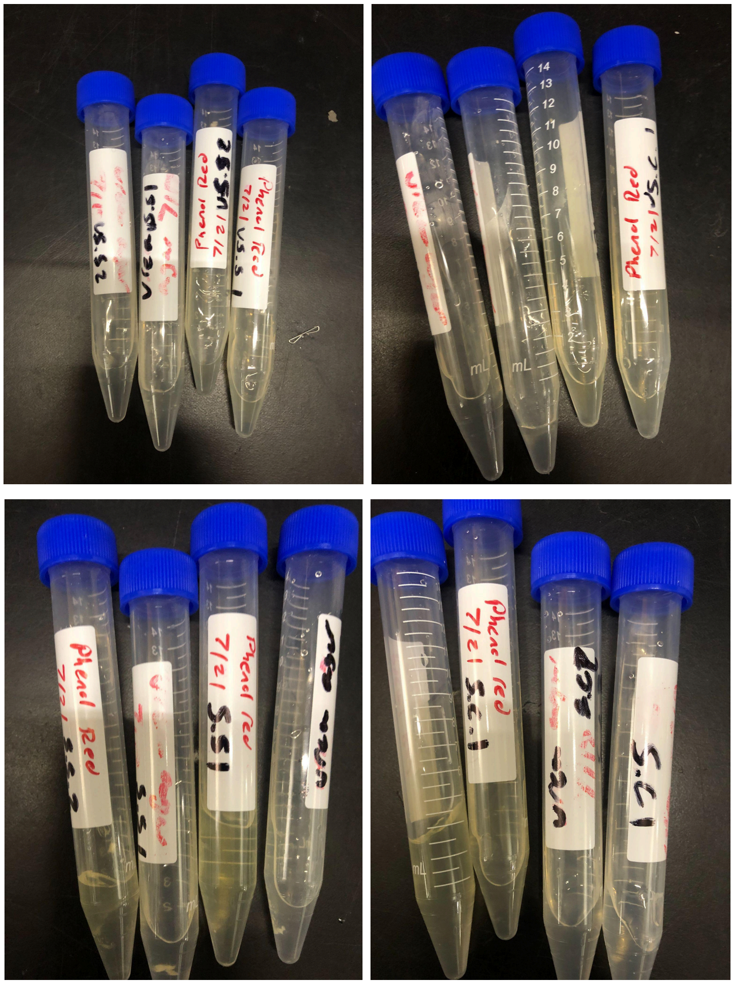

Figure 11: Phenol Red and Urea tests for Unsporulated samples (Top Left), Unsporulated Controls (Top Right), Sporulated samples (Bottom Left), and Sporulated controls (Bottom Right).

To further elaborate, the phenol red and urea tests would -under standard conditions for Bacillus subtilis- produce negative results, indicating that they would ferment a clear color in each vial. Based on the results of the urea and phenol red tests, negative results were indeed produced from both the control and sample groups that were launched, suggesting the idea that no genetic mutation had occurred.

Payload Sensor Data

Data retrieved from the payload's onboard sensor array indicate that operations remained nominal until approximately 50 minutes into the roughly three hour round trip flight time. The temperatures of each of the two samples, control boxes, and their averages are presented as a function of time in figure 12.

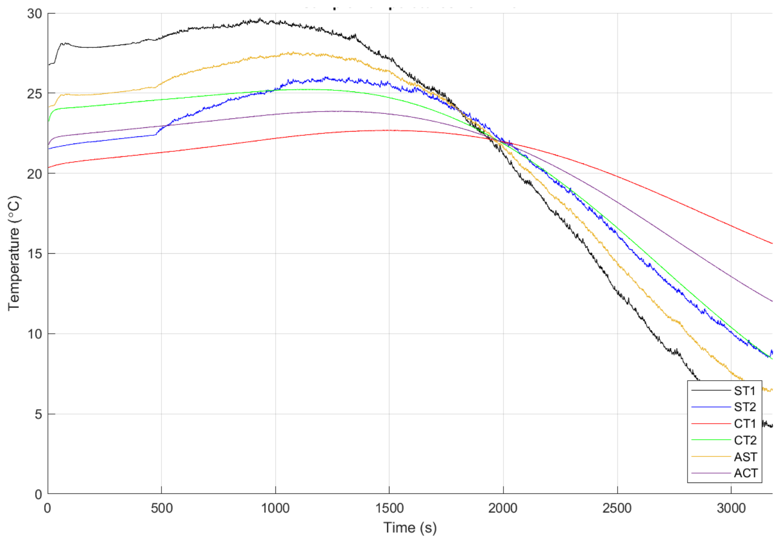

Figure 12: Control, Sample and Average Temperatures

These results are consistent with what is anticipated from literature as temperature decreases with altitude within the troposphere due to the decrease in air density ("Layers of the Atmosphere").

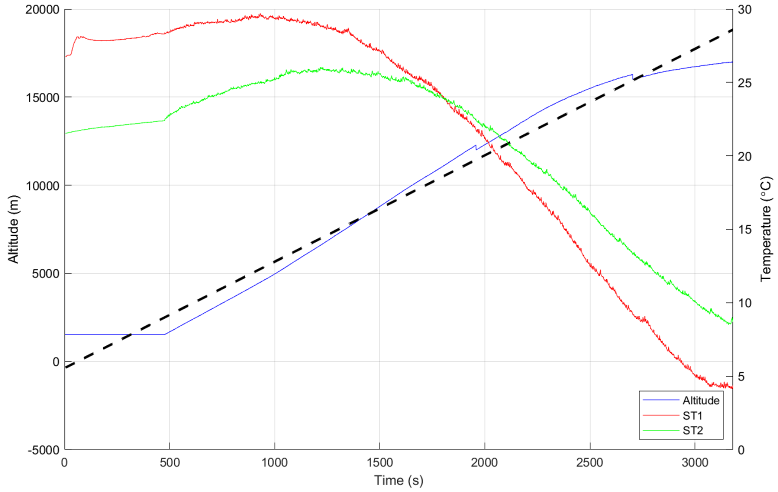

Figure 13: Altitude with Sample Temperatures

This behavior is further reflected in the decrease in pressure and humidity shown in figure 14 & figure 15.

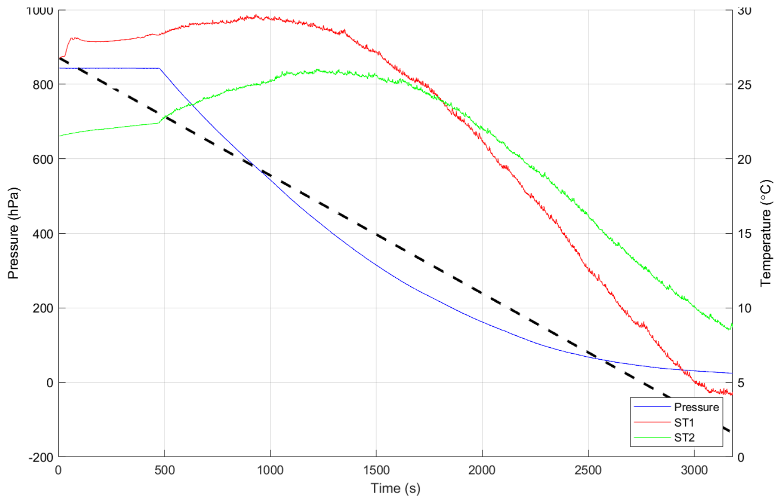

Figure 14: Pressure with Sample Temperatures

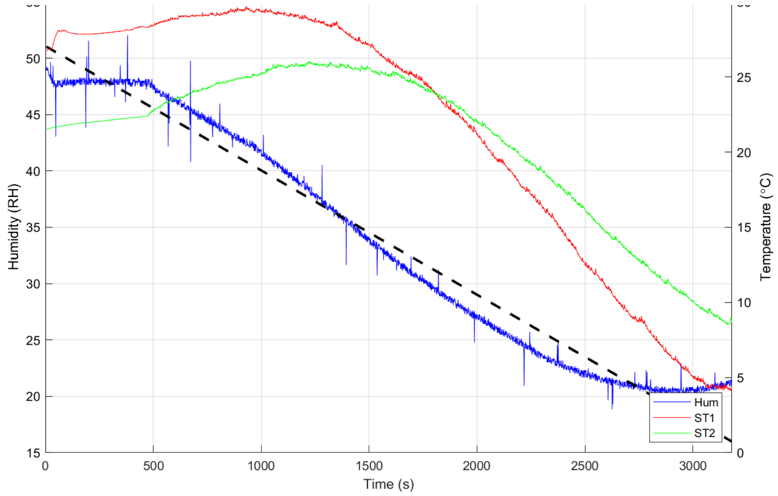

Figure 15: Humidity with Sample Temperatures

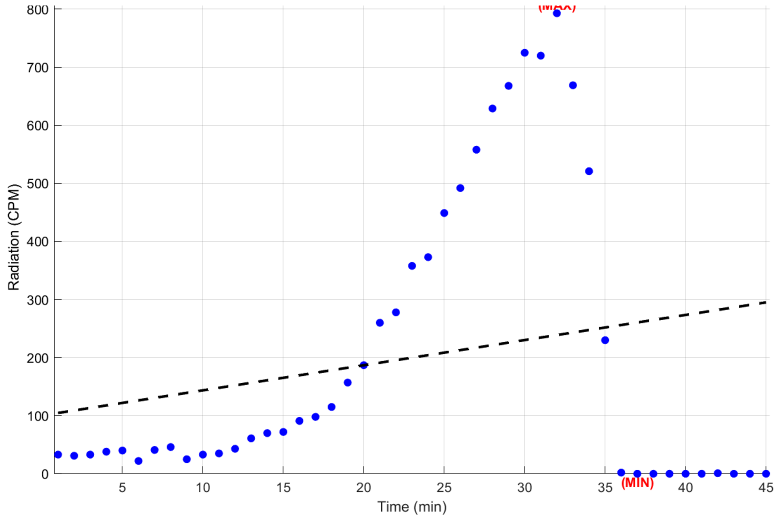

Figure 16: Radiation (Counts Per Minute)

Conclusion

It is believed that low temperatures and low pressures caused an electronic failure as the payload passed through the ozone layer. As shown in *Figure 22* the radiation has a steep upward slope that generally is in agreement with what is expected during the ascent. However, it took a very drastic drop at around 32 min after reaching its maximum value of 793 CPM and then leveled out at 0 CPM which reflects a catastrophic electrical failure. It is theorized that improper sealing of the electronics compartment lead to overheating of electrical components due to the low air density in the upper atmosphere. After retrieval, the payload was stuck indicating that it was in the process of writing data to the SD card (as indicated by external LED indicators). However, turning the device off revealed that data had been saved successfully and post retrieval testing lead to results indicating nominal operation. Future iterations of such an analysis would benefit greatly from ensuring that the electronics compartment is properly secured and air-tight prior to launch.

Despite this hiccup in data collection the other data points show data that was to be expected during this leg of the trip. The highest altitude recorded by the payload was around 56,000 ft just over half of the maximum 102,000 ft. Humidity dropped as low as 20% (relative) which was also expected at this altitude. Pressure dropped a bit more drastically than initially thought as pressure readings were as low as 2400 Pa at the end of data recording. This could have been due to a calculator error from the sensor or unforeseen atmospheric conditions. It is thought that this factor could have contributed to the Geiger counter failure as the vacuum test had shown concerns regarding operation in lower conditions though this had been believed to be resolved.

Furthermore, results show that both the sporulated and unsporulated samples of subtilis survived during the 3 hour flight. However, bacteria density per sub colony was significantly higher for the sporulated samples vs unsporulated samples, indicating that the sporulated samples survived better than the unsporulated samples. Additionally, due to the limited time frame of the experiment, 3 hours was not enough time to allow any reproduction to successfully occur for either of the bacteria colonies, especially given the harsh atmospheric conditions. Therefore, the urea, MR and MacConkey tests resulted in a lack of change between the samples and the control groups, indicating no mutations occurred.

Acknowledgements

- Dr. Azer Yalin - Resources and project design/schedule guidance
- Mech Department and the Idea2Product lab at CSU - The tools to print and manufacture our payload
- Jacob Gottfried - Providing constructive criticism in addition to aiding in project insight
- Seth Antozzi - Providing constant resource and material gathering as well as aiding in payload design and idea construct
- Genesis Marrero - Aiding and assisting in cell culturing, cell management, and all bacteria testing
- The DEMOSAT Space Pigs - Comradery

Message to Next Year

If you're using biological samples, make sure you look up and address any safety hazards and biological risks associated with the samples you are using. Additionally, a lot of the heavy work that our group faced was addressed and completed early on, providing the remainder of the 10 week summer with a low stress environment. Therefore, it is encouraged that any future groups reading this should get as much preliminary work regarding research and construction done early, so the remainder of the work schedule is lax. Finally, choose a project that you are truly interested in, instead of a project you think will be easy. By being interested in your project, you'll have pride in the work you do, and be encouraged to further push the limits of your abilities instead of providing mediocre work that you won't be nearly as proud of.

You may think your project insignificant in the grander scale of things, but the reality is that science is not often perpetuated by the sudden brilliance of a single individual. Science is a crawling process, pushed by those who dare to be creative when presented with the opportunity. Pursuing interests in the things you're passionate about may someday provide the inspiration for another. We all build on the works of our predecessors, and by building upon those works you too can add to the collective knowledge of humanity.

This is a great opportunity to showcase your accumulated knowledge and expertise you may have developed over your undergraduate program. This project will test your rigor, poise and dexterity both mentally and socially through development, testing and launch. Learn the ins and outs of your own thinking and get to know your colleagues to promote healthy cooperation and problem solving in the development of your payload. Successful projects are produced by intense and constant collaboration both with your superiors and coworkers.

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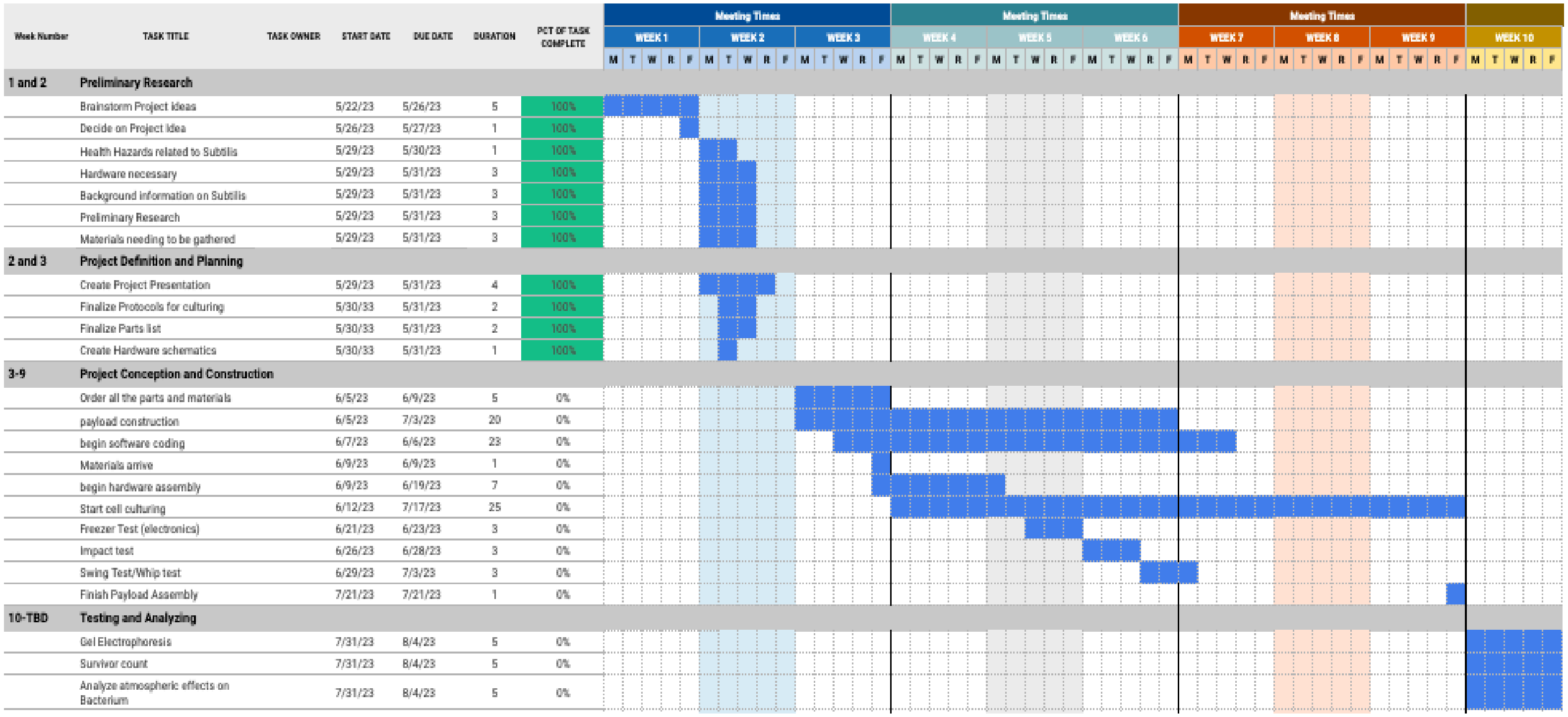
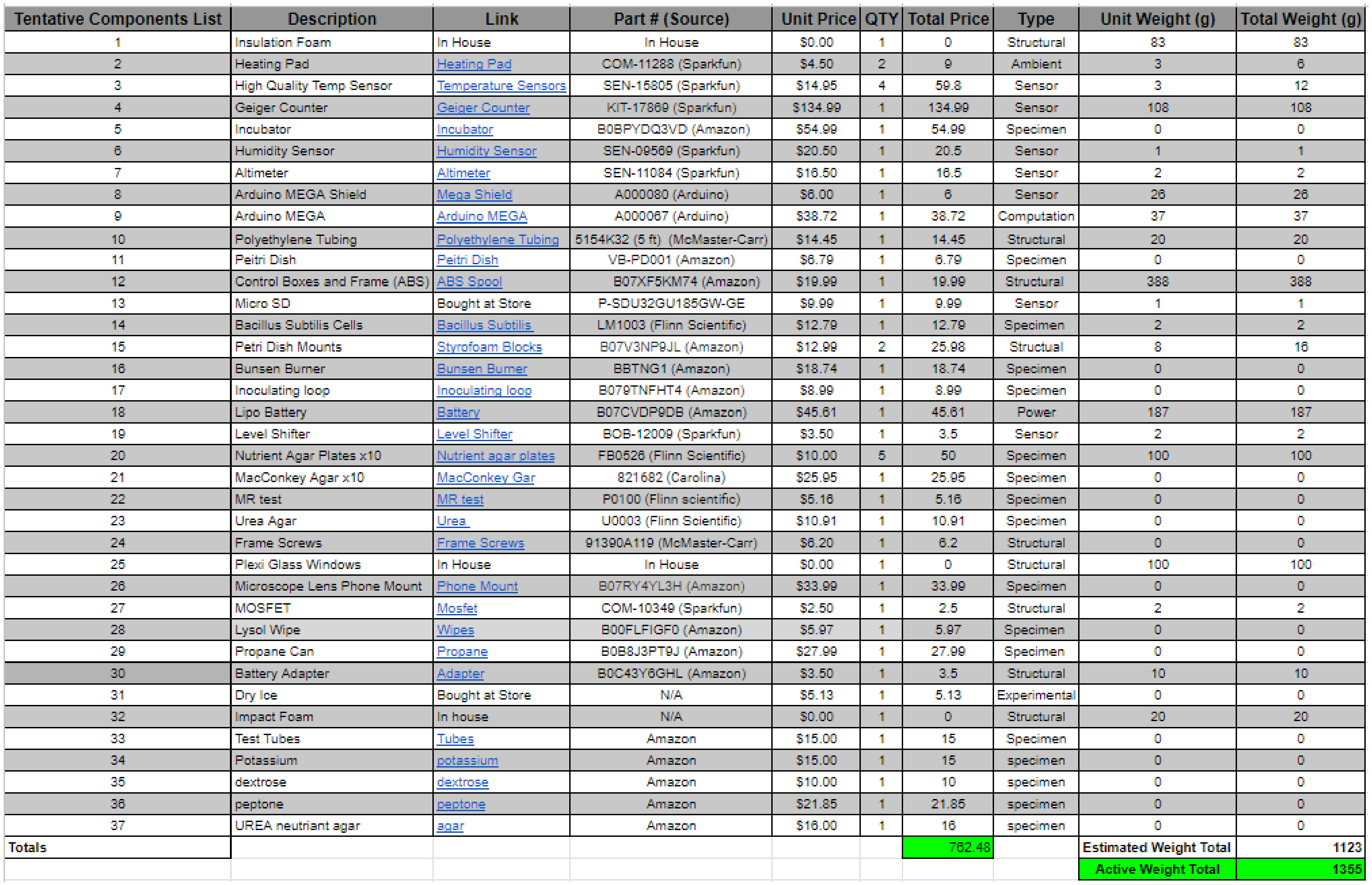
Appendix A - 10-Week Project Schedule



Week Number	TASK TITLE	TASK OWNER	START DATE	DUE DATE	DURATION	PCT OF TASK COMPLETE
1 and 2	**Preliminary Research**					
	Brainstorm Project ideas		5/22/23	5/26/23	5	100%
	Decide on Project idea		5/26/23	5/27/23	1	100%
	Health Hazards related to Subtilis		5/29/23	5/30/23	1	100%
	Hardware necessary		5/29/23	5/31/23	3	100%
	Background information on Subtilis		5/29/23	5/31/23	3	100%
	Preliminary Research		5/29/23	5/31/23	3	100%
	Materials needing to be gathered		5/29/23	5/31/23	3	100%
2 and 3	**Project Definition and Planning**					
	Create Project Presentation		5/29/23	5/31/23	4	100%
	Finalize Protocols for culturing		5/30/33	5/31/23	2	100%
	Finalize Parts list		5/30/33	5/31/23	2	100%
	Create Hardware schematics		5/30/33	5/31/23	1	100%
3-9	**Project Conception and Construction**					
	Order all the parts and materials		6/5/23	6/9/23	5	0%
	payload construction		6/5/23	7/3/23	20	0%
	begin software coding		6/7/23	6/6/23	23	0%
	Materials arrive		6/9/23	6/9/23	1	0%
	begin hardware assembly		6/9/23	6/19/23	7	0%
	Start cell culturing		6/12/23	7/17/23	25	0%
	Freezer Test (electronics)		6/21/23	6/23/23	3	0%
	Impact test		6/26/23	6/28/23	3	0%
	Swing Test/Whip test		6/29/23	7/3/23	3	0%
	Finish Payload Assembly		7/21/23	7/21/23	1	0%
10-TBD	**Testing and Analyzing**					
	Gel Electrophoresis		7/31/23	8/4/23	5	0%
	Survivor count		7/31/23	8/4/23	5	0%
	Analyze atmospheric effects on Bacterium		7/31/23	8/4/23	5	0%

Appendix B - Total Project Expenses and Budget

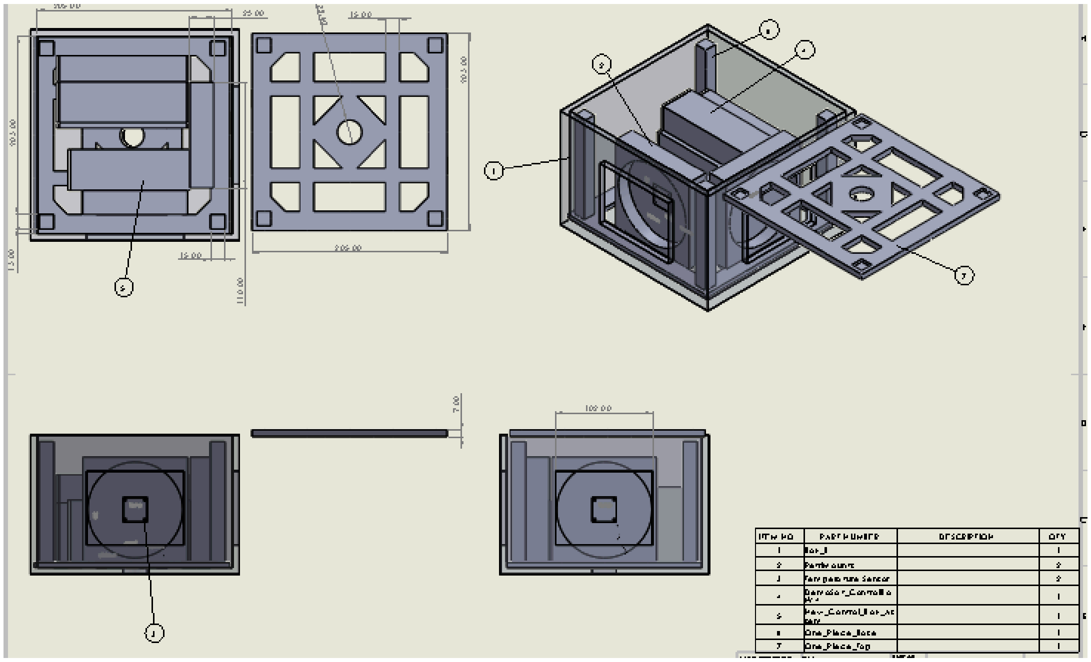


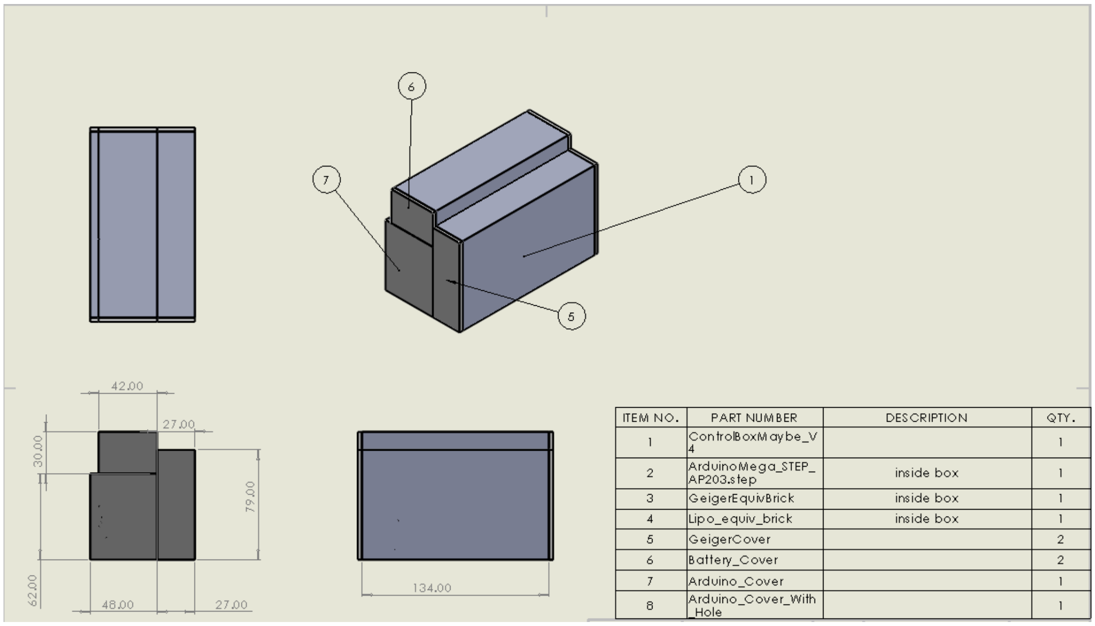
Tentative Components List	Description	Link	Part # (Source)	Unit Price	QTY	Total Price	Type	Unit Weight (g)	Total Weight (g)
1	Insulation Foam	In House	In House	$0.00	1	0	Structural	83	83
2	Heating Pad	Heating Pad	COM-11288 (Sparkfun)	$4.50	2	9	Ambient	3	6
3	High Quality Temp Sensor	Temperature Sensors	SEN-15805 (Sparkfun)	$14.95	4	59.8	Sensor	3	12
4	Geiger Counter	Geiger Counter	KIT-17869 (Sparkfun)	$134.99	1	134.99	Sensor	108	108
5	Incubator	Incubator	B0BPYDQ3VD (Amazon)	$54.99	1	54.99	Specimen	0	0
6	Humidity Sensor	Humidity Sensor	SEN-09569 (Sparkfun)	$20.50	1	20.5	Sensor	1	1
7	Altimeter	Altimeter	SEN-11084 (Sparkfun)	$16.50	1	16.5	Sensor	2	2
8	Arduino MEGA Shield	Mega Shield	A000080 (Arduino)	$6.00	1	6	Sensor	26	26
9	Arduino MEGA	Arduino MEGA	A000067 (Arduino)	$38.72	1	38.72	Computation	37	37
10	Polyethylene Tubing	Polyethylene Tubing	5154K32 (5 ft) (McMaster-Carr)	$14.45	1	14.45	Structural	20	20
11	Peitri Dish	Peitri Dish	VB-PD001 (Amazon)	$6.79	1	6.79	Specimen	0	0
12	Control Boxes and Frame (ABS)	ABS Spool	B07XF5KM74 (Amazon)	$19.99	1	19.99	Structural	388	388
13	Micro SD	Bought at Store	P-SDU32GU185GW-GE	$9.99	1	9.99	Sensor	1	1
14	Bacillus Subtilis Cells	Bacillus Subtilis	LM1003 (Flinn Scientific)	$12.79	1	12.79	Specimen	2	2
15	Petri Dish Mounts	Styrofoam Blocks	B07V3NP9JL (Amazon)	$12.99	2	25.98	Structual	8	16
16	Bunsen Burner	Bunsen Burner	BBTNG1 (Amazon)	$18.74	1	18.74	Specimen	0	0
17	Inoculating loop	Inoculating loop	B079TNFHT4 (Amazon)	$8.99	1	8.99	Specimen	0	0
18	Lipo Battery	Battery	B07CVDP9DB (Amazon)	$45.61	1	45.61	Power	187	187
19	Level Shifter	Level Shifter	BOB-12009 (Sparkfun)	$3.50	1	3.5	Sensor	2	2
20	Nutrient Agar Plates x10	Nutrient agar plates	FB0526 (Flinn Scientific)	$10.00	5	50	Specimen	100	100
21	MacConkey Agar x10	MacConkey Gar	821682 (Carolina)	$25.95	1	25.95	Specimen	0	0
22	MR test	MR test	P0100 (Flinn scientific)	$5.16	1	5.16	Specimen	0	0
23	Urea Agar	Urea	U0003 (Flinn Scientific)	$10.91	1	10.91	Specimen	0	0
24	Frame Screws	Frame Screws	91390A119 (McMaster-Carr)	$6.20	1	6.2	Structural	0	0
25	Plexi Glass Windows	In House	In House	$0.00	1	0	Structural	100	100
26	Microscope Lens Phone Mount	Phone Mount	B07RY4YL3H (Amazon)	$33.99	1	33.99	Specimen	0	0
27	MOSFET	Mosfet	COM-10349 (Sparkfun)	$2.50	1	2.5	Structural	2	2
28	Lysol Wipe	Wipes	B00FLFIGF0 (Amazon)	$5.97	1	5.97	Specimen	0	0
29	Propane Can	Propane	B0B8J3PT9J (Amazon)	$27.99	1	27.99	Specimen	0	0
30	Battery Adapter	Adapter	B0C43Y6GHL (Amazon)	$3.50	1	3.5	Structural	10	10
31	Dry Ice	Bought at Store	N/A	$5.13	1	5.13	Experimental	0	0
32	Impact Foam	In house	N/A	$0.00	1	0	Structural	20	20
33	Test Tubes	Tubes	Amazon	$15.00	1	15	Specimen	0	0
34	Potassium	potassium	Amazon	$15.00	1	15	specimen	0	0
35	dextrose	dextrose	Amazon	$10.00	1	10	specimen	0	0
36	peptone	peptone	Amazon	$21.85	1	21.85	specimen	0	0
37	UREA neutriant agar	agar	Amazon	$16.00	1	16	specimen	0	0
Totals						762.48		Estimated Weight Total	1123
								Active Weight Total	1355

Appendix C - Cell Culturing Protocols/Test Recipes

Cell Culturing Guide can be acquired here: [Cell Culturing]
Urea recipe can be acquired here: [Urea Recipe]
Phenol Red recipe can be acquired here: [Phenol Red]

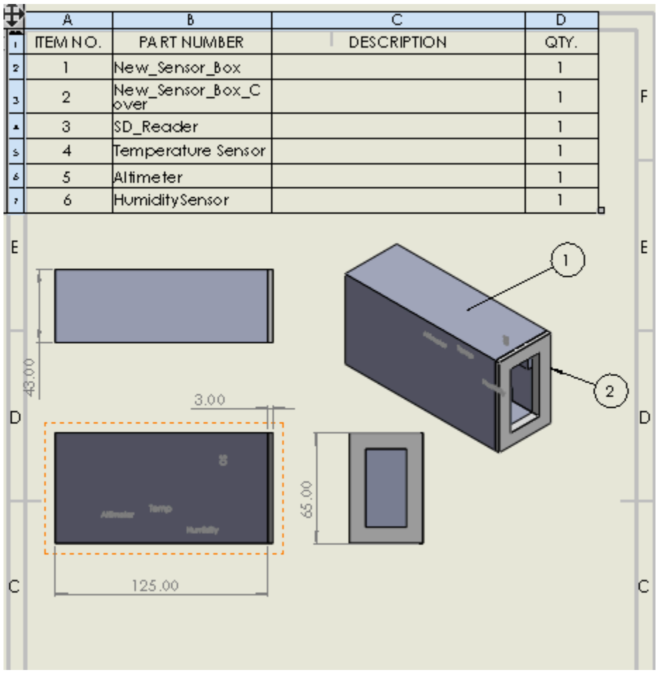
Appendix D - Payload Schematics





ITEM NO.	PART NUMBER	DESCRIPTION	QTY.
1	ControlBoxMaybe_V4		1
2	ArduinoMega_STEP_AP203.step	inside box	1
3	GeigerEquivBrick	inside box	1
4	Lipo_equiv_brick	inside box	1
5	GeigerCover		2
6	Battery_Cover		2
7	Arduino_Cover		1
8	Arduino_Cover_With_Hole		1

Control Box 1



ITEM NO.	PART NUMBER	DESCRIPTION	QTY.
1	New_Sensor_Box		1
2	New_Sensor_Box_Cover		1
3	SD_Reader		1
4	Temperature Sensor		1
5	Altimeter		1
6	HumiditySensor		1

Control Box 2