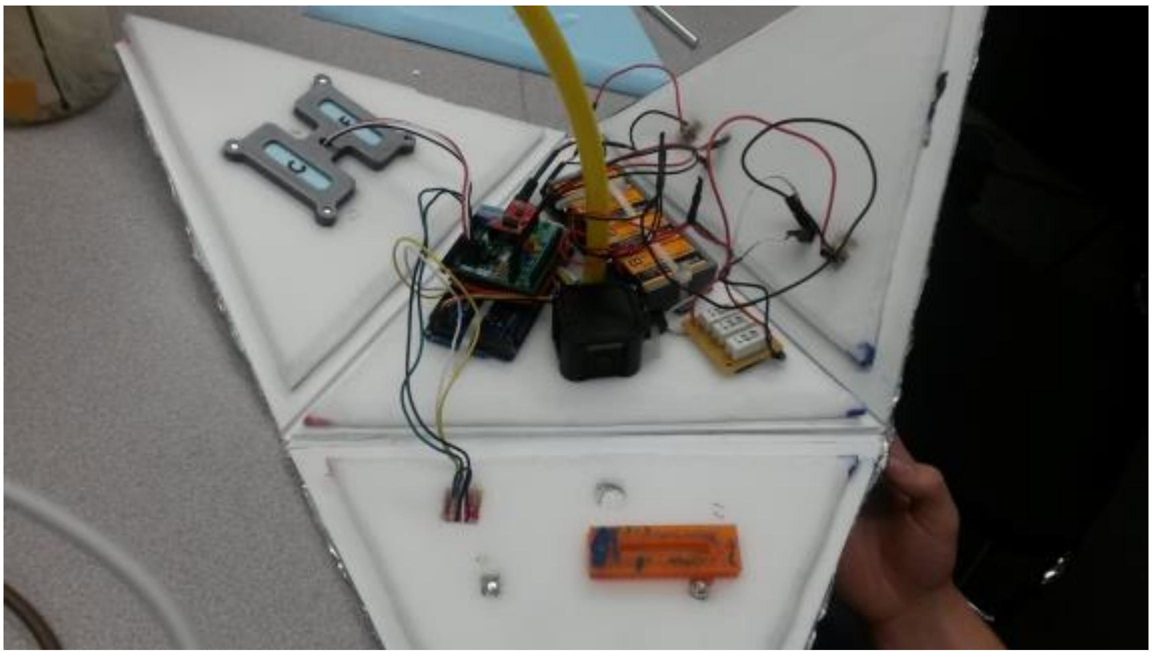
Colorado Space Grant Consortium

DEMOSAT
DESIGN DOCUMENT

Red Rocks Community College



Written by:

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1.0 Mission Overview

Our mission originally had two hypotheses. The first was to determine if gradual exposure to extreme low-temperatures over many generations increases yeast tolerance to low temperatures. The second was to document the difference between natural Ultraviolet Radiation in the stratosphere versus germicidal lamps effects on *Halobacterium salinarum*. Both biological samples can help determine if similar microbial cells could be capable of surviving conditions in a Martian environment.

Recently, several studies have been done to evaluate the presence of microbes in the upper atmosphere (Hume). Microbes surviving in the harsh conditions of the stratosphere have implications in both the ability of microbes and pathogens to transfer across continents. Survival of microbes in very harsh conditions could have implications in the potential survival of microbes on Mars. Thus, adapting microbes to harsh conditions suggests our ability to colonize and terraform Mars. To gain further understanding of the tolerance of microbes to conditions in the stratosphere, the ability of the yeast *Saccharomyces cerevisiae* to withstand freezing temperatures can be tested. Experimental evolution is an established technique used to adapt *Saccharomyces cerevisiae* to variable environmental conditions including drug resistance and ethanol tolerance (Dunham). Serial transfer is known to change both the physiology and genetics of yeast. To evaluate if the yeast subjected to the freeze-thaw cycles has evolved to tolerate the low temperatures of the stratosphere live cells are counted before and after space flight with the cold adapted strain vs. a control strain. Physiological adaptations to the fermentation process of the yeast subjected to freeze thaw cycles and space flight sustained will also be evaluated.

Exposing Halophilic archaea to a UVB-UVC radiation in the stratosphere and a germicidal lamp in lab is related to Mars because Mars surface contains high levels of UV radiation. *Halobacterium salinarum* are resistant to UVB and UVC more than other halophiles. Antioxidants molecules in the archaea resist the DNA-damaging effects. The idea of this experiment was to determine the ability to survive in the stratosphere.

2.0 Requirements Flow Down

Level 0 requirements	**Purpose**
Yeast	Determine difference in tolerance from gradual exposure to low temperature
Level 1 requirements	**Purpose**
Arduino	Data Collection
Temperature and UV sensor	Data Collection
Cold Bath	Flash Freezing
UV Light	UV exposure
Heater	Functional equipment

3.0 Design

The outer design of the payload is a three-sided pyramid (as seen in Figure 1). This was because it is the strongest shape and it allowed for multiple sides faced upward with large surface areas. This was helpful because each experiment could utilize one entire side of the pyramid.

The yeast was placed in an H-shaped 3D printed holder on the side farthest from the heater in order to be exposed to the cold. The two sides of the H held the samples and the center held the temperature sensor, which was through the foam board exposed to the outside temperature (as seen in Figure 2). Centrifuge tubes with threaded lids were used during launch to keep everything dry and uncontaminated.

The Halophile was placed in a quartz cuvette held in a 3D printed holder with a slit through the foam core to expose to it UV (as seen in Figure 3). It was kept close to the heater in the design to narrow the experiment to one variable.

The RRCC COSGC Eclipse Team also asked us to place seeds on the outside of our payload as test experiment for them. They will be experimenting with the effect on seeds when exposed to all conditions in the stratosphere. The seeds were attached to the outside by a 3D printed frame holding a mesh fabric in place and a UV sensor placed nearby. Both the halophile and seeds were placed on the same side. The third upward facing side was designated to the controls and indication lights (as shown in Figure 4).

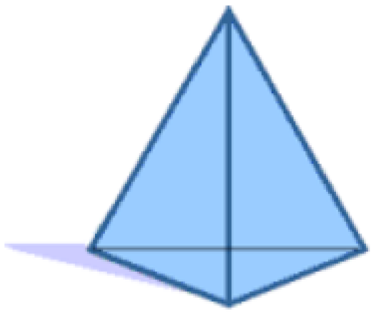


Figure 1

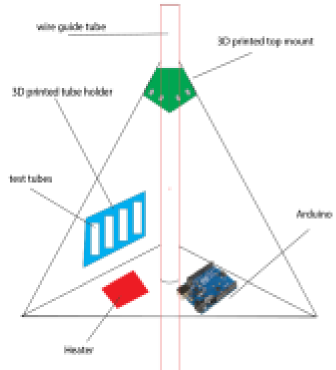


Figure 2

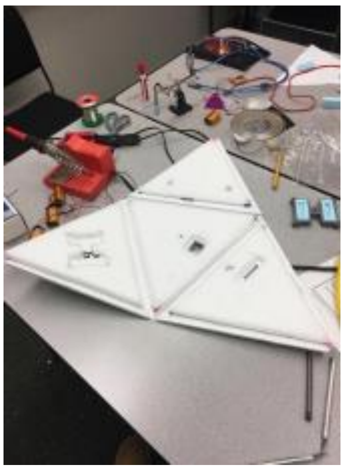


Figure 3

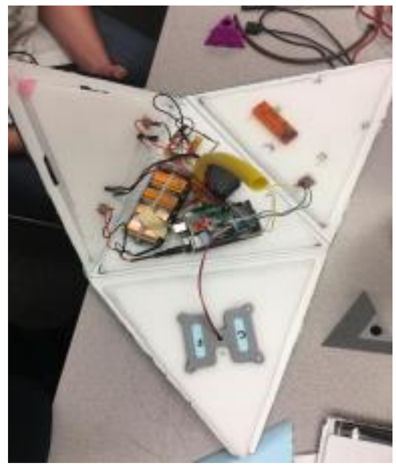


Figure 4

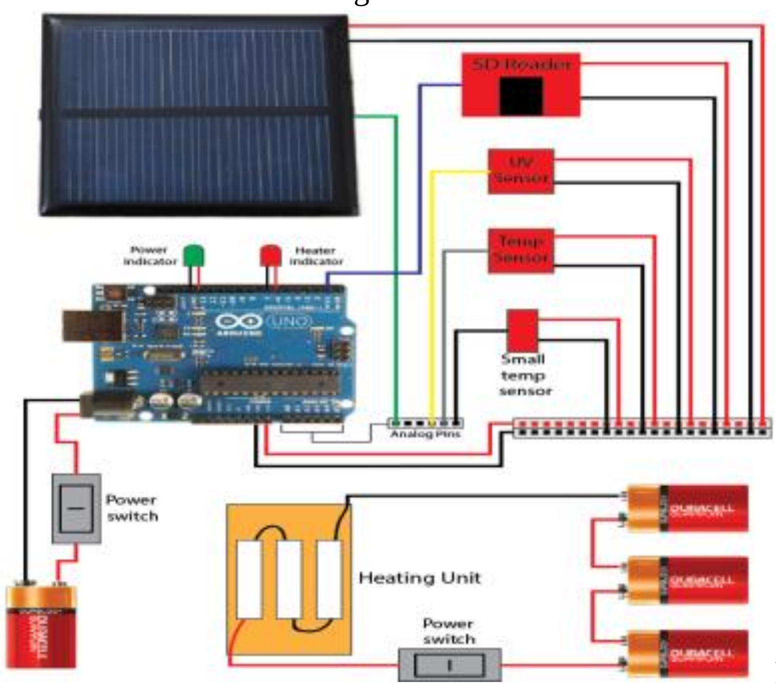


Figure 5

(note the solar panel was included in an early block diagram and not included in the final)

3.1 Parts List

Parts	Mass (g)
U.V Radiation Sensor	3.0
Centrifuge Tube (6x)	52.2
Quartz Tube (4x)	37.5
Temperature Sensor (2x)	3.2
Thick plastic tube	12.6
Insulation and Foam Core	400 Appx
Arduino Uno/Mega (as Package)	56.3
Seeds	100
Yeast (saccharomyces cerevisiae)	1.0
9V Battery 4x	184.28
Hardware (screws, tape, etc.)	100 Appx
Heater	38.3
3D printed Parts	90 Appx
(Note max weight given = 1,000g)	Total: 978.38 g

4.0 Management

Below, the participants are listed by their specific involvement. Our advisors are listed as our go-to for confirmation we are on the right path. Advisor Lynn albert gave us tremendous help and insights about the microbiology aspect of the mission. Sami and Annie took the lead in narrowing down our experiment after brainstorming sessions. Sami and Somair focused on the *Halobacterium salinarum* (cancelled on 6th april, see page 11-13 for explanation). Annie, Donny and Kelsey flash froze the yeast. Everyone helped with the hands-on things that need to be done and attended meetings to be on the same page as a group. Forest thought of the payload design and did all programming and 3D printing. Forest, Kelsey and Annie worked together to construct the payload itself. As a whole, each member played a part to help each idea grow into a successful product.

Faculty Advisors-
Barbara Sobhani, Lynn Albert, Brandon English

Members-

Annie Strange	Team Lead
Forrest Havermann	Design and Programming
Annie Strange, Kelsey Hoon and Donny Herrera	Biology (Yeast)
Sami Tariq and Somair Taj	Biology (Halobacterium Salinarum)

5.0 Budget

Parts	Company	Price
U.V Radiation Sensor	N/A	N/A
Centrifuge Tube (6x)	N/A	N/A
Quartz Tube (4x)	AdValueTech	$80
Temperature Sensor (2x)	N/A	N/A
Thick plastic tube	N/A	N/A
Insulation and Foam Core	N/A	N/A
Arduino Uno/Mega (as Package)	N/A	N/A
Halobacterium Salinarum	VWR	$12
Yeast (saccharomyces cerevisiae)	N/A	N/A
9V Battery 4x	N/A	N/A
Dry Ice	King Soopers	$50
Hardware (screws, tape, etc.)	Home Depot	$10-15
Heater	N/A	N/A
3D printed Parts	RRCC Idea Lab	N/A
YPD	VWR	$70 Total $227

6.0 Test Plan and Results

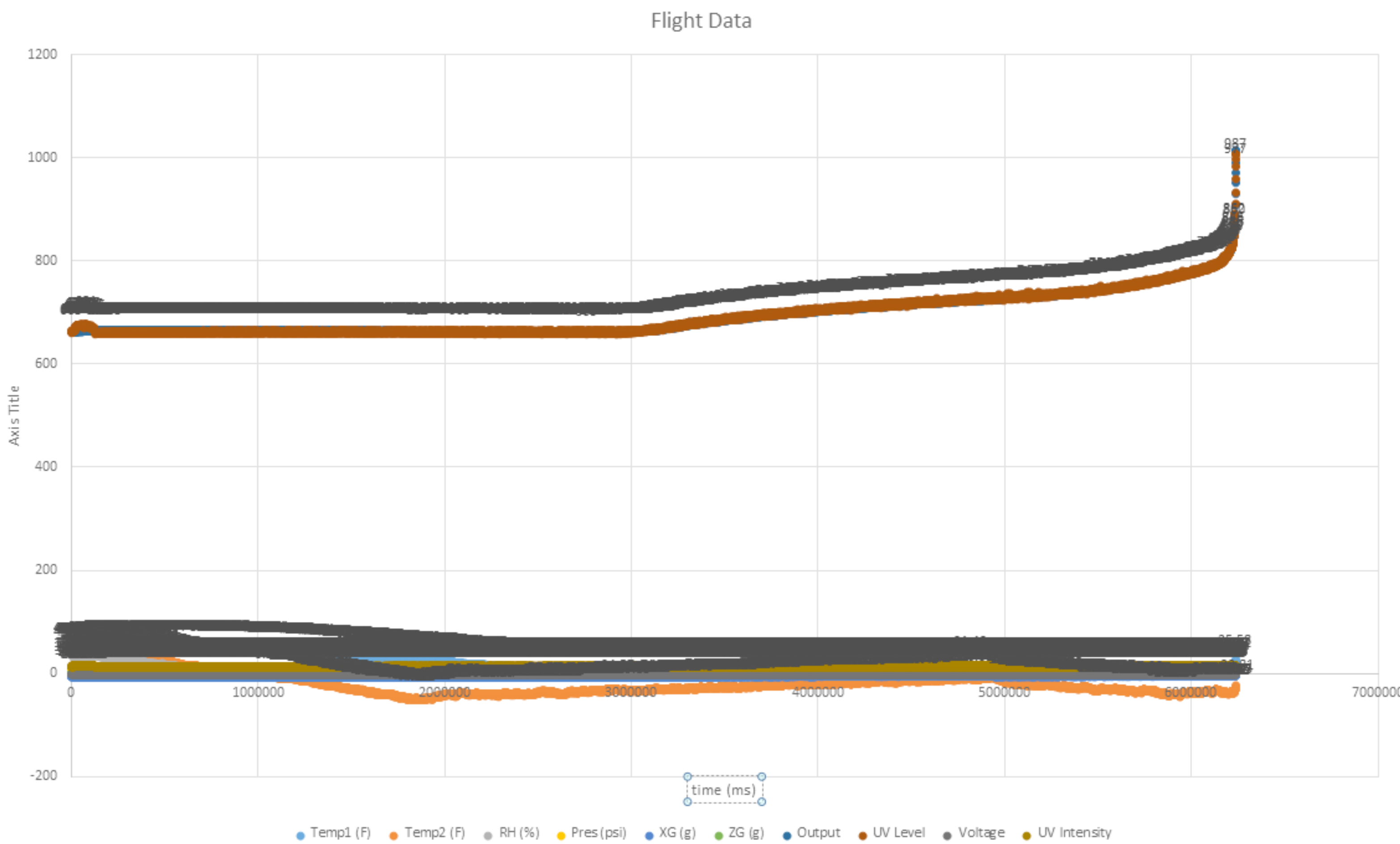
To test the payload there was multiple drop tests, rapid movement tests and an arduino functional test sure that the items inside the payload were ready four launch. The drop test was done from two stories and there were minor dents and scratches on the payload. There was also a stair test done to ensure when knocked around everything would be secure and once again minor damage was done to the inside of the payload. The GoPro shifted slightly but come to find out the camera battery did not hold a charge so that did not end up being an issue. There was also a whip test done where it was tied to a string and yanked vigorously multiple times. A cold test was also done where the payload was placed in a freezer for two hours with all systems running. The containers that hold all samples were pressure tested and impact tested as well as cold tested to assure they would withstand flight. Unfortunately, the cuvette that was meant to hold the halophile cracked at the top. This made it impossible to put the halophile in the cuvette in liquid form therefor it had to be inserted dry which could have caused some damage to the microbe. A functional test was done with the arduino running for two hours and successfully recorded everything correctly. All test insured the payload was ready for flight.

7.0 Expected Results

There were three separate experiments going up on the payload so there were three separate expected results. For the yeast, gradually exposing eukaryotic cells through generations may heighten their chances of survival in certain conditions therefore it was predicted that the cold adapted yeast will survive the space flight in higher numbers than the control. For the halophile, the question being asked was is an ideal place for testing biological hypotheses about Martian habitability or more specifically Martian radiation? It was expected that damage in the *Halobacterium salinarum* exposed to germicidal UVC (in lab) and the halophile exposed to stratospheric UV would be higher than the control sample in the payload. For the seeds, the eclipse team is going to attempt to germinate them after the launch exposure. This will be compared to the exposure during the eclipse launch. It was expected that the seeds exposed to more radiation during our flight would be less likely to produce germination and growth than the seeds exposed during the Eclipse flight. If any germinate and grow this will allow for more growth time to examine the effect on the plant from the exposure.

8.0 Launch and Recovery

The day before launch the samples were prepared by plating, spinning down the required amount for the pellet and the supernatant was removed from the pellet of both the frozen and the control. The samples were placed on ice and transferred to a fridge (around 0ºC) until launch day when they were placed in a cooler and transferred to the payload. Forrest was the payload handler and carried it off to flight. After landing, the samples were rehydrated with YPD in the field and placed back in the cooler. The samples were then transferred to the microbiology laboratory and serial dilutions between 10^{-1} and 10^{-5} were plated. The yeast colonies will be counted allowing us to compare the effect of space flight on cold-adapted yeast vs. control yeast.



9.0 Results, Analysis, and Conclusions

It is predicted that the cold adapted yeast will survive the space flight in higher numbers than the control. The CFU (colony forming unit) was counted for the cold-adapted strain after the in lab freezing and the control strain, providing an estimate on how many cells survived the freezing process. The CFU counts from the frozen strain before the launch was 1.3×10^{-6} before freezing and 1.2×10^{-6} after freezing showing that most of the cells survived the freezing process.

From the flight data, the lowest temperature reached on the inside of the payload was 0.33°C and the lowest temperature reached on the outside of the payload -42.7°C. As these temperatures typically pose a survival issue for yeast, the next step was to plate and count colonies of the control and cold-adapted yeast cells before and after the balloon flight. It was predicted a higher portion of the cold adapted strain would survive space flight than the non-adapted yeast cells.

The data produced interesting results that proved the hypothesis incorrect. First of all, it was found that the strain used seemed to be tolerant to cold temperature to begin with (as seen in Figure 1). That is something that was concluded from the first freezing. This ability to survive after freezing may have been higher because the supernatant was removed before each freezing. Secondly the frozen strain died more during flight than the control strain (as seen in Figure 2), which could be for a couple of different reasons. During the freeze thaw cycles the cell could have been damaged, making the damage on the control strain during flight minimal compared to the frozen strain. In the future, additional variables such as susceptibility to radiation in the upper atmosphere might need to be considered.

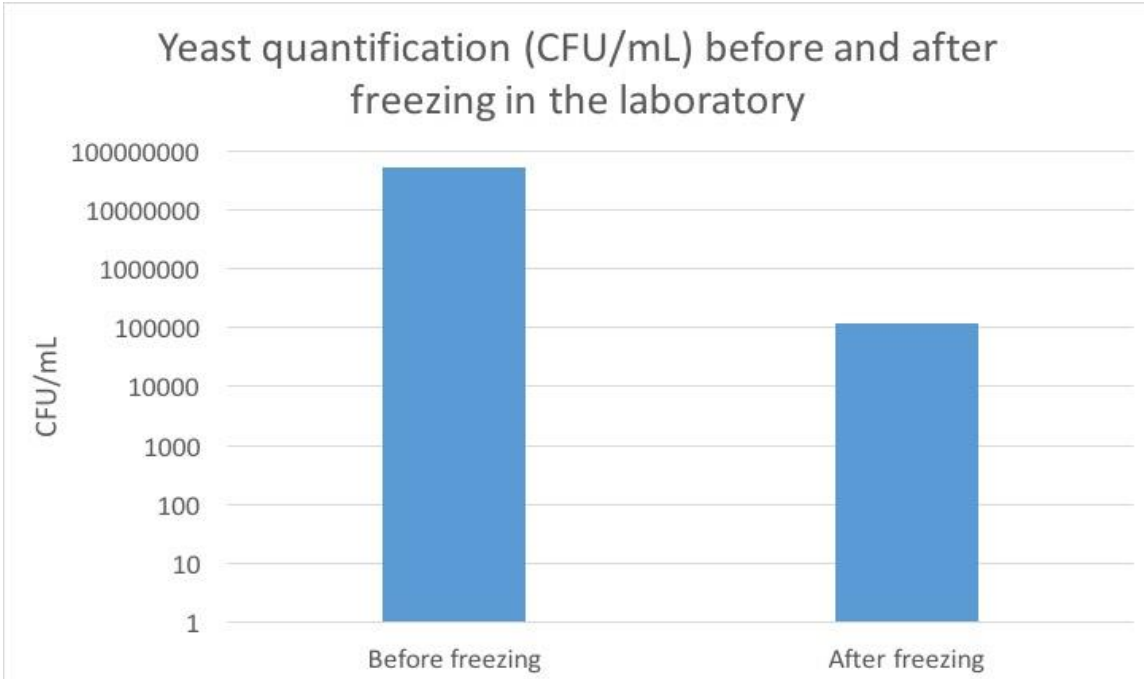


Figure 1. Quantification of yeast before and after a freeze-thaw cycle in the laboratory.

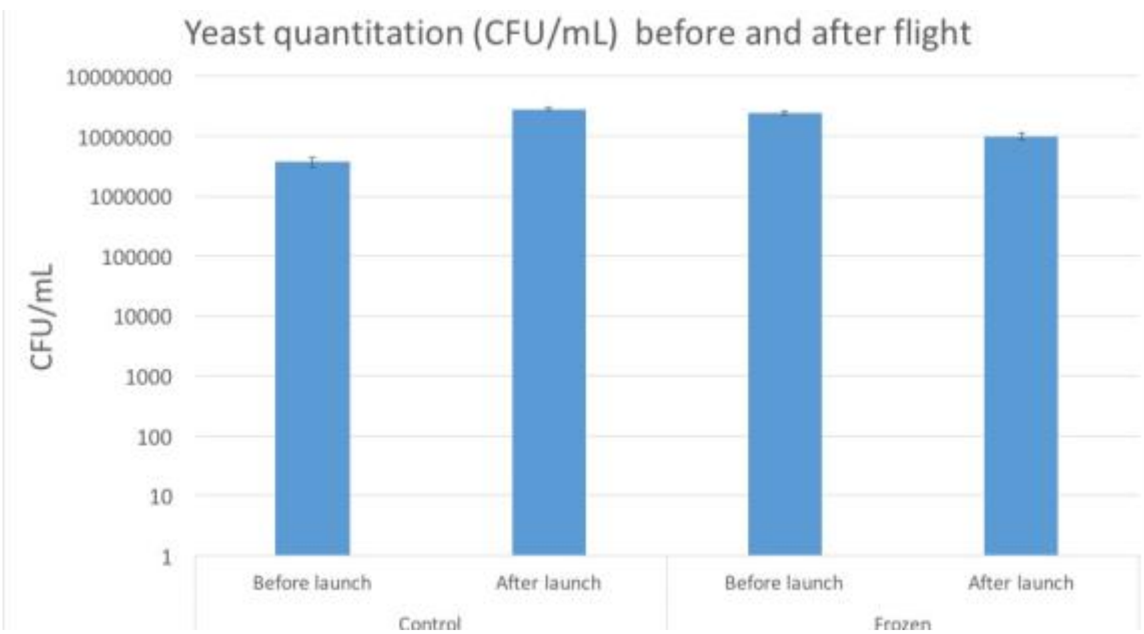


Figure 2. Quantification of control and cold-adapted yeast before and after balloon launch.

10.0 Ready for Flight

If this payload were to fly again the camera needs to be replaced, but otherwise it wouldn't need any improvements. It would only need a new experiment.

11.0 Conclusions and Lessons Learned

It was initially planned for the payload to carry two samples the yeast (*Saccharomyces cerevisiae*) and the halophile (*Halobacterium salinarum*). There is much information available on the exposure of microbes using germicidal lamps, germicidal lamps operate usually with one wavelength like 254nm of UVC. The sun is not a lamp that operates on a singular wavelength, the sun produces the entire UV spectrum, UVA UVB and UVC, there is little data available on percentage of wavelengths and intensity of UV in the stratosphere. The calculations were not done for this experiment to have a known value for the exposure that the halophile flown underwent. This was due to the inability to obtain a usable sample for flight.

All other aspects of this launch were fully executed and were able to produce results concluding a disproven hypothesis for the yeast. The seeds are being grown and the first to sprout have been Broccoli and Kale. More data will be concluded from that experiment coming after August 2017.

13.0 Message to Next Year

Annie:
Teamwork makes the dream work ☺

Sami:
I think it would been very beneficial to me if I planned all the details involved earlier and identified all the unknown components, if there are little scientific research done on particular subject before that means there is a good opportunity and many questions need to be answered but it also means a lot of unexpected delays, to get maximum accuracy from DemoSat experiment, I would've needed more extensive research in all the different components involved to assess whether or not an idea is practical for DemoSat.