Searching For Bacteria At High Altitudes Using Silicone Media & Polymerase Chain Reaction Gene Amplification

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April 27th, 2019

Abstract

Microbiology in the atmosphere has been a growing field and has some interesting implications on the ubiquity of microbes and their intercontinental spread. The field has some inherent difficulties associated with it especially when it comes to collecting data. Having an affordable and re-usable collection system that can expose samples to upper atmospheric conditions could improve the range and quality of studies that aim to examine ecosystems and weather patterns of these altitudes. We designed a payload that conceals a collection medium that can resist low temperature and pressure environments and only exposes the payload at certain pressures. We found a relatively cheap and re-usable method that, with a few design changes, could fill this need.

1. Introduction

Microbiology in the upper atmosphere is new and exciting field of study that aims to answer questions such as intercontinental microbial exchange & viability of pathogens in the atmosphere. The means of data collection is difficult both from the perspective of accessing the upper atmosphere and mitigating contamination as the payload falls through the troposphere.

There is a study where a balloon with a payload of similar nature was tethered approximately 500 ft up *Allison M Spring et al*. This collection method has advantages in time of exposure and control in recovery of the samples however is limited by its range of collection. A tether can only be built so long and there is likely key data at much higher altitudes.

Another study where a jet was used which is highly is effective at gathering substantial amounts of bio-mass for genetic sequencing. This collection method has a high likely hood of contamination from the surface of the jet and non-intended altitudes and is limited in its accessibility and cost *DeLeon-Rodriguez et al*.

2. Methodology

We used a two-sheet aluminum panel sliding door to catch microbes from the upper atmosphere. One panel had four wells that held a silicone gel that was resistant to low temperatures and pressure. The sliding door had four windows that corresponded to these wells and were off set until the intended altitudes. A high torque servo motor was used to move the sliding door because of the high friction caused by the dow corning grease that acted as our Gasquet material. The code used in an Arduino recorded temperature, pressure and humidity as well as control the servo motor. A heater was integrated to keep all the parts warm enough to operate. The servo motor was set to open forty minutes after the Arduino was turned on or if the payload reached 3 PSI. It was set to close after a following twenty minutes or if the payload reached 5 PSI after opening. To further asses weather the door opened a piece of sun paper was placed under the door. If the paper is exposed to UV radiation It will change color from dark blue to a lighter blue. If the strip of paper remained dark then the door did not open, if it has a lighter blue section the width of what the door should have opened than it opened.

After the payload was recovered Several swabs of the outside surface and inside wells were taken with sterile Q-tips and placed in sterile tubes. These samples were then sent through a PCR reaction to amplify the DNA and test for the presence of prokaryotic microbes. A following PCR reaction that targets Bacilli was run to test for the presence of a specific genus of bacteria. Testing was done by running the amplified DNA down an agarose gel to measure and visualize and products and compare them to a molecular ladder that would give a relative size of the DNA fragment.

3. Results

After the payload was launched it reached a height of approximately 95,000 ft. After we recovered our payload and took our samples for testing, we observed that the sun paper indicated that the sliding door successfully opened and closed (Figure 1). After analyzing the data from the SD

card we found that the Arduino turned on and off several times and our timer did not work. The pressure switch turned out to be the determining mechanism as the code would continuously read the command to open at our intended altitude range. Luckily the code also included a closing mechanism in case of an early burst and became the means the payload used to close and remain closed. There was a miss read or some other error and the code shows that the door opened for a few minutes after landing (Figure 2). This is a significant confound that puts doubt into our biological results.

Our first round of test did not find any presence of bacteria however our positive controls also came back empty which tells us that PCR failed. The second round of test gave us a reliable result. Our Gel showed no presence of prokaryotic DNA but our negative and positive controls came back negative and positive respectively (Figure 3). This suggests that there is either a lack of sufficient prokaryotic DNA or our wells did not come in contact with any microbes.

4.Conclusion

After considering the confounds of this experiment there are several points where improvement could be had. Due to the limited surface area, exposure time of the payload and density of bacteria at these altitudes there may not have been enough time for sufficient biomass to accumulate on the collection surfaces. A redesign that focuses on maximizing surface area or using an air pump/ filtration system may prove to have more chance of success. Due to the nature of weather balloon testing there is not a means of maintain a height for a long period of time and will have to rely solely on surface area.

In order to prevent a malfunction on the ground during recovery it would be safer to include a switch that would turn off the Arduino or control mechanism after the door closed. Any exposure after landing immediately casts doubt into the origin of the samples and collection would be best if done under a bio-hood.

The use of sterile swabs also turned out to be inhibitory as they absorbed the silicone medium used for collection and made the biological test quite difficult. The use of a sterile scraper would likely provide more reliable samples.

Limitations in our time and access to tools and space restricted our ability to conduct a full analysis of the collected material and determine the efficacy of our control collection well. The genetic material amplification test showed that it is difficult to obtain enough DNA for PCR as we had a small window of exposure on the ground and still had a negative result. This suggest that a method where the collection medium is allowed to grow in a medium like a nutrient broth will better test for a microbial presence and provide enough material to preform genetic identification.

5. References

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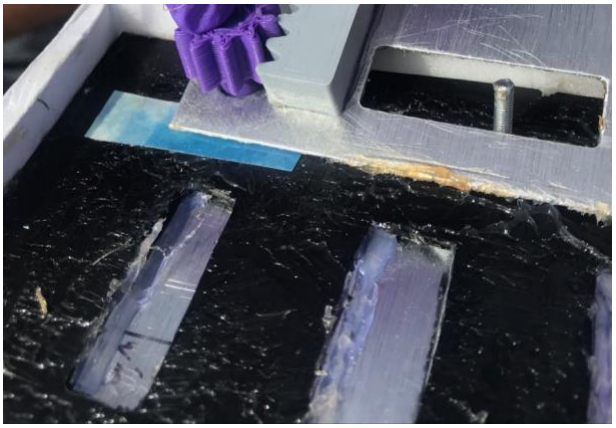

Figure 1: Sun paper test
The presence of a light blue band shows that the door opened and exposed that area to UV light. The distinct line from light blue to dark blue suggests that there was no means for UV reach the area intended to remain unexposed and give us a false positive.

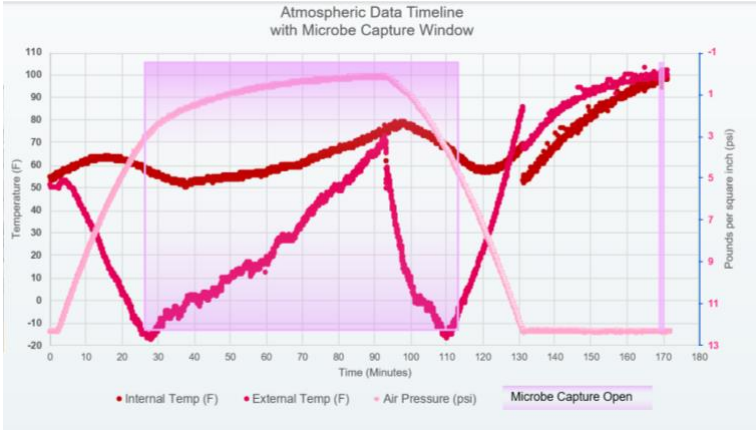

Figure 2: Data from flight sensors and door exposure time.
This chart shoes the change in temperature inside and outside, the pressure and the time frames in which the sliding door was open. We see a temperature pattern that matches what we would expect when rising through the troposphere and stratosphere. The door opens and closes right as the temperature vector changes and suggests that the door was open only in the stratosphere with the exception of the small open and close at the end.

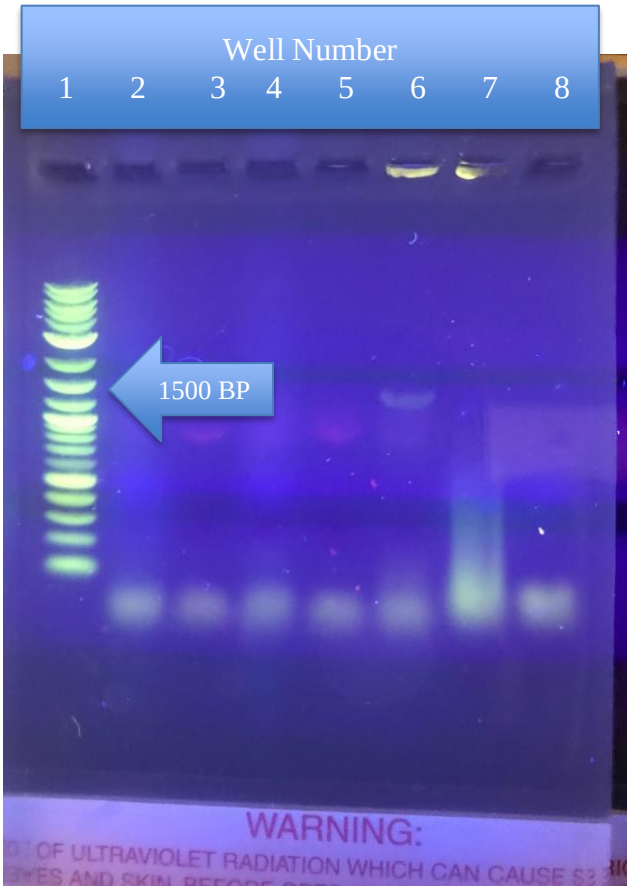

Figure 3: Gel electrophoresis of the genetically amplified material.
The gel shows that there was no presence or substantial enough genetic material for genetic amplification. One of our positive controls (well 6) gave a positive reading and all the other wells cam back negative including our second positive control (well 7). The second positive control was given a large number of bacteria and likely interfered with the reaction. Wells 2 and 3 were swabs of the outside of the sliding door and wells 4 and 5 were of the inside of collection surface 1. Well 8 is a negative control. Well 1 is a molecular ladder by which to compare the length of the bands against.