SOCIETY OF PHYSICS STUDENTS
An organization of the American Institute of Physics

SPS Chapter Research Award Proposal

Project Proposal Title	High Altitude Rocket Assisted Micro-Organism Capture (HARAMOC)
Name of School	The University of Tennessee - Knoxville
SPS Chapter Number	7158
Total Amount Requested	$2000

Abstract

The Society of Physics Students at the University of Tennessee Knoxville is seeking to use a high powered transonic rocket to collect high altitude bacteria and viruses known as bioaerosols. These poorly understood microorganisms may play an important role in cloud seeding and atmospheric chemical processes which have climate implications.

Proposal Statement

Overview of Proposed Project

There is a question of what life exists off our planet. Astrobiology seeks to answer that question by examining extremophiles here on earth. The purpose of this experiment is to examine microorganisms and viruses that live in the middle to upper troposphere, collectively referred to as bioaerosols. The identity and role of these organisms is currently unknown, but it is believed they may play a role in cloud seeding and even climate change. These organisms have likely adapted to sustained airborne life, but as of yet their growth needs and ecological niche have yet to be determined.

Previous studies have been unable to collect samples under sterile conditions. The prior experiments have relied on gondolas, which are carried under a giant balloon that was inflated on the ground, mountain top collection where the units are lying on the ground nowhere near unadulterated air, and from hurricane case planes whose samples are highly contaminated by material ripped into the upper atmosphere by storms as they inch towards the land. Carbon-14 analysis in these limited studies indicates that these organisms live out their lives high above the ground.

We plan to build sterile collection payloads for a sounding rocket to be tested in the spring in Kentucky and then launched in the Spaceport America Cup competition in June in New Mexico. These payloads will sample approximately 1-2 cubic meters of air collecting between 5-10 thousand individual microbes per flight, although the microbial population density is not well-understood and will be a part of our exploration. For the 2020 Spaceport America Cup, the payload will be flown to 30,000 feet and will collect an order of magnitude more sample.

Once the collection has been made the sample will then be divided in half, with one part being sent off to a sequencing lab and the other half will be plated on various types of agar to grow as many of the acquired organisms as possible. The half sent for sequencing will first have to have viable DNA samples extracted. Then up to 47 samples per flight will undergo 16S Amplicon Sequencing through a v2500 cycle.

This project pulls personnel, resources, and facilities from Physics, Chemistry, Microbiology, Ecology and Evolutionary Biology, Aerospace Engineering, and Electrical Engineering. This work will pave the way for other interdisciplinary work and help to expand interest in our growing Biophysics subfield. It will allow new physics students to get involved and make connections with experts and future experts across the sciences and engineering.

Background for Proposed Project

Bioaerosols are believed to play an important role in the Earth's climate, one example being the possibility of these aerosols seeding cloud formation; however, not much is known about microbial life suspended in the upper troposphere [1]. Current sampling has been limited to either mountain-based passive collection stations or atmospheric balloons [2,6]. Aircraft have been used to collect these bacteria but there were issues with ground contamination stirred up by powerful storms [6]. In all of these studies, contamination of samples has proven to be their greatest challenge [1,3]. Our proposed sampling technique employs the use of an experimental sounding rocket which, through transonic flight, will sterilize the outer surface of the payload.

The planned processing of samples collected by this sounding rocket involves gene sequencing to compare bioaerosols collected during the flight with samples collected on the ground surrounding the launch site [1,3,6]. We will collect our own samples in an electrospun polymer membrane, and by flying in the transonic regime and using aerodynamic heating and high velocity airflow to sterilize the surface of the rocket, we will be able to overcome challenges faced by some of the other studies performed on bioaerosols, an example being a study performed by Smith [5] where the majority of the culturable samples were from the fuselage and not their collection media.

DNA sequencing is an extremely useful tool identify bacteria. For this experiment, we will sequence the 16S rRNA portion of the bacterial DNA. The 16s rRNA gene is a sequence of DNA that encodes for a subunit that is the RNA portion of a ribosome, the protein-producing molecular machine. A portion of this gene is highly conserved across all species of bacteria, with a variable segment specific for each bacterial species. The variable portion allows for ease of identification of the specific bacteria collected. The data taken from this sequencing will be compared against other known 16S rRNA sequences stored in public databases, giving us a definite identity of the bacteria. It is likely some microbes sampled will be new to science and will greatly differ from their terrestrial counterparts due to their extended suspension in air. Next year we wish to examine the possibility of building a low pressure circulated air bioreactor to attempt to grow collected bacteria under similar conditions to their native habitat. Due to time constraints and currently available funding, we have chosen to forgo this option for the time being.

Expected Results

Our initial test will be conducted in Kentucky in early Spring tentatively March 18. We expect our rocket to reach an altitude of 11,000 feet above sea level. At apogee the payload will be deployed to collect microbial samples as it descends to 10,000 feet where it will be hermetically sealed. Thus, it will be able to descend from the troposphere avoiding contamination from other particulates in the atmosphere. The flight is expected to last roughly 187 seconds, and a ground sample at the point of impact will be collected from the ground as a control. The samples will immediately be transported back to the University of Tennessee; from there the samples will be analyzed in the facilities provided by the Department of Ecology and Evolutionary Biology who have been kind enough to provide us lab space, equipment, and expertise. They will analyze our sample using half for Gene Sequencing and the other half to try growing in different agar media.

Once 16s rRNA gene sequencing is completed, the results will be put into the BLAST database in order to compare the genetic material against known, and previously sequenced, organisms therefore allowing us to identify the species of the bacteria with a high certainty of accuracy. Since we are unsure of the species of microbes we can expect to find at altitude, any collected samples will be divided out onto either complete or minimal media where we will attempt to grow them under varying temperature and light conditions. It is hoped that at least one growing method attempted will result in amplification of our sample. Our experiment will be considered successful if the DNA from the grown cultures matches the DNA from the gene sequenced filter sample but not control samples taken from the ground, indicating any samples obtained are unique from microbes on the ground and that no contamination has occurred.

Microscopy analysis and possibly further sequencing of the colonies grown will be used to identify what was grown. Comparing this data against the other half that was initially sequenced will not only confirm that the collection was not contaminated between initial sample separation and additional sequencing and will also provide additional data on the tropospheric microbial life. After the test flight our payload will have a second opportunity to collect samples during the competition for the Spaceport America Cup on June 18-22. This launch will serve as a second chance to collect samples, and in the event both sampling attempts are successful, will give us more data about these bioaerosols.

Description of Proposed Research - Methods, Design, and Procedures

Previous studies have used aircraft, balloons, and mountain top collects to recover samples of these microbes but have difficulty reducing contamination. Mountain top capture methods introduce ground contamination. Balloon gondola methods introduce contamination during the filling stage where the balloon is laid out on the ground yet remains above the collection media for the remainder of the flight. The study using hurricane chase planes noted significant amounts of storm blown human and animal waste. For this year's competition the flight will reach a maximum altitude of 10,000 feet which is the lower bound of where these organisms have been reported. By next year our team should have the infrastructure in place to enter into the 30,000 foot competition allowing for an order of magnitude increase in the time of collection of bioaerosols as per rules regarding drogue descent rate for the rocket, there is a minimum descent rate of 75 feet per second before main deployment to ensure that the rocket does not drift onto White Sands Missile Range. Our payload test flight in Kentucky will be able to spend more time at altitude, as we will not have the same constraints regarding minimum descent rate.

After consulting with Prof. Jaan Mannik and Prof. Maxim Lavrentovich in The University of Tennessee Physics Department's two biophysics professors); Prof. Steven Ripp, Professor of Ecology and Evolutionary Biology specializing in microbiology and genomics; and Prof. Kevin Kit, Professor of Materials Science Engineering specializing in electrospun polymers we concluded that it would best to use a commercial off the shelf electro-spun filter element that can perform a size exclusion separation of the desired bioaerosols. While it is possible to purchase borosilicate filters capable of viral capture, they are more expensive and would require a higher pressure gradient maintained to push air across the membrane. The ABS electrospun membranes are ideal for the capture of the full range of microbial life down to 0.2 microns. By only collecting samples larger than 0.2 micron, we will be able to sample a larger amount of air and still be able to capture most microbes.

The payload section of the rocket which houses the filter assembly will be integrated into the Aerospace Senior Design Class's rocket for the Spaceport America Cup. We will use a 3D printed turbine connected to a DC motor capable of spinning a centrifugal pump impeller at 30,000 rpm to achieve a minimum flow rate of 1-2 cubic meters per minute. To prevent the angular momentum imparted to the rocket by the impeller from spinning and tangling the parachute we will have a second centrifugal pump mounted at the top of the payload spinning on the same axis spinning in the opposite direction. While active, the centrifugal pumps will generate a large pressure differential along the center of our canister filter, opening radially mounted check-valves, pulling air through the electro-spun filter and exhausting the air through radial ports. There will be an additional check

valve mounted between each pump and the filter to ensure airflow can only travel one way, through the side of the airframe and out through each pump.

Dr. Steven Ripp and the Department of Ecology and Evolutionary Biology have offered any space, facilities, equipment, personnel and resources needed for analyzing samples we collect. These facilities will be used to sterilize all components by gassing them. Final assembly and sealing of the payload will take place in a clean room, where payloads will be vacuum bagged and stored until launch. We will use these same facilities and room to take the recovered payloads and open them prior to analysis.

The first launch will take place in March in Elizabethtown, Kentucky. Dirt from the base of the launch pad and the landing site will be taken as control samples. This test will also function as a stress test for the payload traveling faster in thicker air, with a longer sustained acceleration than that which will be experienced during launch at the competition. The main will deploy at apogee at 9300 feet and it will collect for 42 seconds, after which the pumps will be turned off and check valves will re-seal. After landing the outside of the payload will be washed with antimicrobial solution and sealed in a vacuum bag. The bag will then be put into a cooler filled with ice to keep the temperature low during the 4-hour drive back to UT's campus. For the first launch we will open the payload in the clean room and cut the filter element lengthwise using half for immediate genetic sequencing and trying various methods of growth media for biological amplification for subsequent sequencing. The competition launch in June will give us additional samples from a new location.

Plan for Carrying Out Proposed Project

- Personnel - As of this moment there are four national SPS members on our team; there are also 12 non-SPS members working on the rocketry team as well. There are 24 additional SPS members that have shown an interest on some level in working on this.
- Expertise – Many of our team members have experience that will prove invaluable to our experiment: Robert Nickel and Peter Tarle both hold Level 2 High Power Rocketry certifications through the Tripoli Rocketry Association and years of experience with high power rocketry, and Maggie Spangler is a senior in microbiology who will be assisting the team.
- Research Space - All research space has been graciously provided by The Department of Ecology of Evolutionary Biology where Dr. Ripp and Dr. Veronica Brown who will conduct all genetic testing and oversee and direct DNA extraction of our sample with our team.
- Contributions of Faculty Advisors - We have three faculty members working closely with us on this project; Maxim Lavrentovich, who serves as our SPS chapter faculty advisor, Dr. Evans Lyne, who is overseeing the design, construction and testing of our launch vehicle, and Dr. Steven Ripp who is assisting us with gene sequencing and consolidation of data once we collect our samples from the payload.

Project Timeline

- Spaceport America Cup Competition Entry Form Submission – November 16th, 2018
- Motor and Payload Preliminary Design Complete – November 17th, 2018
- Competition Team Acceptance Announcement – December 3rd, 2018
- Motor and Payload Final Design Complete – December 15th, 2018
- Prototype Payload and Motor Fabrication – December 15th, 2018 to January 25th, 2019
- First Competition Progress Update Due – January 25th, 2019
- Payload Test Flight Launch Window – February 1st, 2019 to March 31st, 2019
- Flight Ready Motor Completion – March 8th, 2019
- Second Competition Progress Update - March 8th, 2019
- Final Competition Deliverables Due – May 17th, 2019

- SPS Interim Report – May 31st, 2019
- Competition Week – June 17th through 22nd, 2019
- Specimen Analysis – June 24th, 2019 through August 24th, 2019
- SPS Final Report – December 31st, 2019

Budget Justification

Item 1: Donaldson Company 0.2 micron Double Open End Flat Gasket Canister Filter
These filters are the right size to selectively collect bacteria while minimizing the required pressure differential to get the desired flow rate of air. This is the capture media for the experiment and the most critical component. It would be impossible to make such a filter, so we are limited to commercial off-the-shelf canister filters manufactured by the Donaldson Company. In addition, these filters come pre-sterilized in vacuum sealed bags.

Item 2: Tyvek Suits
Tyvek suits will be necessary for processing our payload while in our clean room. In addition, anyone handling the recovered payload prior to final sanitizing and vacuum bagging will wear a Tyvek suit to minimize contamination that could be carried into the clean room back in Knoxville.

Item 3: High-Speed DC Brushless Motor
These motors will power our high efficiency centrifugal pumps. We need to spin the impellers at roughly 30,000 rpm to achieve the desired air flow rate, and to do that without excessive weight, we will be using motors normally used for RC aircraft.

Item 4: MatterHackers Nylon X 3D printer Filament
Our payload will endure a wide range of temperatures and considerable stresses at launch and during operation; because of this, we have chosen to use Nylon X for all 3D printed components. Nylon X is resistant to higher temperatures than both the ABS and PLA normally used in 3D printers and is reinforced with carbon fibers for additional rigidity and strength.

Item 5: 11.4 V, 2200 mAh Lithium-Polymer batteries
The weight constraints we have to work with for our payload mean we need to use light weight batteries with high energy density; LiPo batteries are the only reasonably priced batteries with enough energy density to power our pumps. Each pump can run a full collection cycle on a single 2200 mAh battery, so two batteries will be needed for each payload.

Item 6: Cesaroni Technologies Pro54-6GXL Reloadable Rocket Motor Casing
The motor reloads donated for use in our test-flight requires a 6 grain CTI reloadable motor casing. The casing is a metal shell that encases the actual rocket propellant, closures and nozzle and allows it to be mounted in our expendable rocket.

Item 7: Cesaroni Technologies Pro54 Rocket Motor Aft Closure
The motor casing does not come with a threaded closure, which is required for retaining the propellant grains and nozzle inside of the motor casing.

Item 8: Sterile Respirator Masks
In addition to Tyvek suits, any members inside of the clean room at the time of payload assembly will wear respirators to prevent them from exhaling on to the otherwise sterile filter element.

Item 9 and Item 12: Ice Bags and Cooler

We will need enough ice bags to keep our payload at around 4-5℃ for the duration of transportation from each launch to the clean room where they will be sequenced. This is to minimize the metabolic rate of any captured micro-organisms and improve their odds of surviving long enough to sequence.

Item 10: Sterile Tape

We need sterile tape to seal gloves to Tyvek suits to avoid contamination of the payload prior to launch and of the sample upon recovery.

Item 11: Vacuum Bagger and Vacuum Bags

Vacuum bags will be used to store the payload in a sterile condition prior to launch and returning from sampling mission. The vacuum sealer comes with all the bagging material we would need.

The University of Tennessee Chemistry Department has already supplied gloves, lab space, chemicals, and computational time for catalysis studies to improve rocket motor performance. The Department of Ecology and Evolutionary Biology has already committed to donating lab space, establishing a clean room, and providing the experience and equipment necessary for gene sequencing and growth of any samples we acquire. EEB's contribution is expected to be around $10,000 worth of sequencing services beyond the cost of lab space and sample extraction.

The construction and testing of the rocket has been paid for by the Department of Mechanical, Aerospace and Biomedical Engineering, Imperia Aerospace, the Tennessee Space Grant Consortium and Contrail Rockets, and we are currently pursuing additional funding as well. So far, pledged contributions from these sources have exceeded $19,000. All subsystems have been funded with the exception of the payload.

SPS, Student Space Technology Association, and University of Tennessee Amateur Radio members have loan or allocated over $2500 worth of personally owned equipment towards this endeavor. Including electronics, high power rockets and even a 3D printer. We have also received assistance from the Department of Material Science and Engineering, Department of Mechanical Aerospace and Biomedical Engineering, Department of Chemistry, Department of Ecology and Evolutionary Biology, and from the Department of Physics and Astronomy.

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