I. Cover page

Proposal Title: Are harmless viruses able to infect the Vibrio Cholera Bacteria in a microgravity setting?

Grade Level(s) of Submitting Student Team: Grade 9

Submitting School: Riverside Secondary School

Submitting School District: School District SD43

Submitting Teacher Facilitator: Mr. S Robinson.

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For our experiment, we would like to see the effects of a harmless viruses infecting Vibrio Cholera, because this experiment could be a very important scientific and medical discovery. If the virus does attack the Vibrio Cholera, it could lead to further scientific research, that could help prevent many bad bacteria from infecting our bodies. Imagine what a breakthrough it could be if we could stop bacteria by using harmless viruses. It could definitely create a new aspect in scientific and medical research. The way bacteria reproduce is by binary fission, which means that the DNA is splitting into two exact replicates of the single cell. In other words, the donor cell produces pilus, then brings the 2 cells together, and a strand of DNA is transferred. We would like to learn about bacteria and how it becomes affected by good viruses. Our proposal is to see if the bacteria type Vibrio Cholera is able to produce pili, which is a virus receptor in a micro-gravity setting, and then be attacked by the good virus. This is so that we can eventually learn if good viruses are able to kill bad bacteria in a micro-gravitational setting. The virus we are using is harmless because it does not cause disease in humans, however it should infect the bacteria. The only way for this to happen is if the bacteria is able to produce pili so that the mutualistic relationship between the virus and the bacteria is able to work.

II. Team member page

Co-Principal Investigators (listed in alphabetical order)

Name: Grant, JANNA
Grade level: 9

Name: Harvey, OLIVIA
Grade level: 9

Name: Sunderji, ASHIANA
Grade level: 9

Name: Turner, EVAN
Grade level: 9

Collaborators (listed in alphabetical order)

Name: Miguel Gutierrez

Grade level: Graduate Student, SFU Molecular Biology Biochemistry

III. Experiment materials and handling requirements page.

List of Proposed Experiment Samples (Fluids and Solids to be Used)

- 5ml of Vibrio Cholera
- 5ml of Bacteriophage
- 10ml of Agar plate

LIST YOUR PROPOSED EXPERIMENT SAMPLES ON THE FOLLOWING PAGES FOR THE TYPE OF FME YOU ARE PROPOSING TO USE IMPORTANT:

Are any of the proposed samples human in origin? (check one):

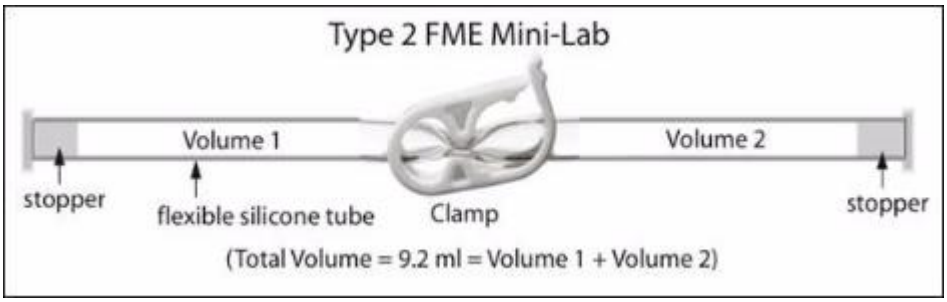
No human samples in origin.

Special Handling Requirements During Transportation

No special handling requirements.

Fluids Mixing Enclosure (FME) Type Proposed to be Used (check one):

Type 2 FME (2 experiment volumes: one clamp used)



Volume 1.

5ml Vibrio Cholera

5ml Agar plate

Volume 2.

5ml of Bacteriophage

5ml of Agar plate

Allowed Crew Interactions	Allowed Modifiers
"Un-Clamp"	During first week – A+2
"Clamp" (means re-clamp)	None
"Shake"	During first week – A+2 Gently shake, 10 seconds
"Wait"	None

IV. The Question to be Addressed by the Experiment

Are harmless viruses able to infect the Vibrio Cholera Bacteria in a micro-gravity setting?

Researchers have found that bacteria grow faster when in a micro-gravity setting (Zhang, 2014). Scientists believe that bacteria have a difference without gravity because of how food and water get transported in and out of the cell. "If you're a microbe, that means all the metabolic waste products and all of the things you breath and eat can only go around by diffusion. The mechanism of transport is very different," states Russel Neches, (Zhang, 2014) The micro gravitational environments also affect the bacterial gene expression, which is when the information from a gene is combined or composed. The bacteria will also spend a lot less time adapting to the micro gravity condition and will concentrate on staying stationary. The bacteria will react like this because of the decrease in mass transfer due to the lack of densities in molecules because of micro-gravity. (Zhang, 2014) All of these cases are indirect effects because they are the cause of extracellular fluid; which is exterior fluid on the cells. (Cauz, n.d.)We believe that with this type of bacteria the effects will be very similar, so that we are able to infect it with the virus (Orlova, 2009). We would like to see what the effects of micro-gravity have on good viruses infecting the Vibrio Cholera bacteria. The bacteria Vibrio Cholera is able to produce diarrhea, dehydration, pain in the abdomen and vomiting. The disease can cause many problems in children if not treated, as well as pregnant women, it can even cause fetal death (Frieden, 2014). We are interested in discovering if bacteria are able to produce the virus receptor, pili, in space.

The pili are the outermost structure of bacteria. The role of the pili is to attach itself to other surfaces, basically creating a bio-film. A bio-film is a group of microorganisms where cells stick to each other and are likely to adhere to host surfaces. If the biofilms are missing a substrate, which is a molecule that acts upon other accelerated molecule or enzyme and microbes, then the biofilm will not form. With gravity pili is produced by the donor cell producing it and linking to the recipient cell. The barrier is nicked so that the DNA is able to get into the recipient cell making both viable donors of pili. Once the bacteria have reproduced pili it is able to be "attacked by the virus" because the pili act like a virus receptor (Garret, Bhakoo, & Zhang, 2008). The name of these viruses that attack bacteria are called bacteriophages, phages for short. Phages are considered mobile genetic particles, that are able to reproduce bacteria using foreign cellular resources.

The understanding of the concept of the harmless viruses killing bacteria is studied today. There is even a lab at the Simon Fraser University where they work with the harmless virus to infect the Vibrio Cholera. Miguel Gutierrez has said that in the lab the virus and the bacteria have a mutualistic relationship that both benefit from their interacting, because when the virus infects the bacteria, it keeps it alive by giving it genes to allow it to survive. The virus benefits because they are attached to an antibiotic virus which provides it with tools to make more viruses. (Gutierrez, 2016)

It is important that we conduct this experiment so we can not only understand the differences between the effects of microgravity versus gravity when harmless viruses are affecting bacteria, but also so that we can better understand the effects of gravity in the production of pili. When the experiment is sent into the microgravity setting, we will be able to identify what parts of this process were led by gravity and what parts of the bacteria and harmless virus produce pili as well as if the harmless virus infects the Vibrio Cholera in microgravity. It will also help us to recognize how gravitational and non-gravitational settings affect harmless viruses and how they are able to reproduce using a living organism, in our case bacteria. We feel that if it works better in a micro-gravity setting, researchers could try to mimic the setting to make the harmless virus infect the bacteria used. It could help scientific research and have the possibility to lead into further research for stopping deadly viruses on earth and/or in space.

V. Experiment Design

Our group has chosen to use samples of the Vibrio Cholera bacterium, and we will be testing it to see if it will create a virus receptor known as pilus or pili in microgravity. Pilus, is grown from bacteria and allows it to adhere to host surfaces around it such as other bacteria. This would let the bacteria commence auto agglutination. Auto agglutination is the clumping of individual bacteria cells and this would be important so that the bacteria can attack the virus. Scientists have found that bacteria grow much faster in micro gravity than on earth, so we are now testing to see if the bacteria will grow pili any faster, and if the good virus can still attack the Vibrio Cholerae in the micro gravitational setting. Our group has chosen this bacterium due to the fact that researchers have found that pili grow on Vibrio Cholerae. (Gutierrez, 2016) If we know that it will grow down here on Earth, then we know that there is a chance that it will grow the same in a micro-gravitational setting. We think that it will grow faster as previous studies have shown that bacteria can grow faster in space. (Zhang, 2014)

The Experimental Materials:

We will need 5ml of the bacteria as well as 5ml of the virus, 10ml of agar plate to feed the Vibrio Cholera and the bacteriophage and the tube provided by SSEP. We chose this specific bacteria (Vibrio Cholera) and the virus (Bacteriophage) because we knew that they would be accessible for our usage. We also know that bacteriophage is a harmless virus, and would suit are experiment as the harmless virus has an important part in our experiment.

Our team's main source of experiment samples will be provided by Miguel Gutierrez. Miguel is a graduate student of Simon Fraser University and is currently studying Molecular biology and Biochemistry; he has offered us samples of the Vibrio Cholera bacteria. We have emailed Miguel and have decided upon an experiment that would be possible with the samples provided generously by him.

Miguel has given us the details of the Bacteria and we now have sufficient information to conduct our experiment without any danger or harm coming to anyone. The bacteria we have chosen does not have side effects that put us or anyone else that will be handling it in any danger. Therefore, this experiment sample will not require any extra forms for it to pass the flight safety inspection.

Procedures:

The first step in this experiment when it goes into microgravity is to set up the container so that it is all prepared for the experiment. We need to put 5ml of Vibrio Cholera on one side of the tube, as well as part of an agar plate to feed the Vibrio Cholera. Then we have to add a clamp at the halfway point of the tube. We need to fill up the rest of the container with the 5ml of Phage. Then up into space it goes. We have requested that the astronaut unclamps the tube 1 week after arriving at the International Space Station. (Roman, 2016) Then the two substances will meet. We have requested that the tube be lightly shaken, for 10 seconds. Because we have given time for the pilus to grow, once they meet, the harmless virus will attack the bacteria, by reproducing inside of it, killing off the disease and manipulating its gene.

Ground elements:

On earth, the experiment will be conducted in the same manner as it will be conducted in the micro gravitational state. We will have 5ml of Vibrio Cholerae, and 5ml of the good virus, as well as on either side we will have a bit of an agar plate, to feed the bacteria. We will have the Vibrio Cholerae in one section of a test tube container, and the good virus in the other side. We will keep the two separate for a week and then will mix them together. Once they are together, we will observe and see if the good virus will infect the Vibrio Cholerae and what reactions will occur, We will be observing the growth of the Pili and bacteria in the micro gravity state, too see if the growth of Pili is accelerated. We will compare the ground experiment as well as the experiment in micro-gravity. We will see if both were affected, if neither were affected or if only one was affected. This will be done by counting the amount of bacteria before and after it has been in Microgravity, and comparing that to the experiment done on the ground. We will record the bacteria count, by using the direct viable counting method (Xu, Roberts, & Singleton, 1982). The direct viable counting methods is a common way to count bacteria growth. The bacteria with the agar plate will be divided into quadrants, and one quadrant will be put under a microscope to be studied. It is one of the most efficient ways of counting bacteria. We will also be comparing the amount of bacteria killed by the good virus when analyzing our results. We will conclude and analyze all the information that we gather. The experiment will show how Vibrio Cholerae is affected by a good virus, as well as all its reactions and how it would change the growth of pili. Both the experiment on Earth and in a micro gravitational setting will play an important part to science research. If proven that the harmless virus can affect and neutralize the bacteria, it could result in further studies about stopping bad bacteria. The results could end up being an important change if we could stop bad bacteria using harmless viruses. The experiment will be the same in the micro gravitational setting, other than that there will be no gravity. We hypothesize that the pili will be grown shortly after the two samples are mixed. The purpose of our ground experiment is to determine the difference between the pili grown on the ground, and the pili grown in micro gravity, as well as stopping the bad bacteria with a harmless virus. This will also help our group determine the speed that the pili are grown, and the differences in how much time it took. Because of the previous studies that have been done on bacteria in space, we think it would be important to see the reaction of Vibrio Cholerae fighting a good virus on Earth, as well as in space. If successful on Earth, it could help in many ways as it would be a better and a more efficient way to eliminate bad bacteria.

Experimental analysis:

When we measure out the substances by using a graduated cylinder, we will need to make sure that we have the exact same amount of substance in the experiment going into space and the experiment that stays on Earth. When we conduct the experiment on Earth, we will need to compare both experiments, finding the how the pili grew and how the harmless virus affected the Vibrio Cholera, to really find the most accurate answer to our question; *Are harmless viruses able to infect the Vibrio Cholera Bacteria in a micro-gravity setting?* Our final analysis will include our most important findings and all of the information of the two final results. A graph and diagram of the results found with the experiment including all of the notes and observations

made during this experiment about pili growth and the amount of bacteria killed, will be included in our final analysis. It is very important that we are as precise as possible while doing the direct viable counting, as well as recording the amount of pili growth, that way our results can be clear and accurate. Finally, our analysis will include any further questions we have that have been brought to our attention through this experiment. We are hypothesizing that the bacteria will produce pili faster and denser, and that the harmless virus will neutralize the Vibrio Cholera, and infecting it much faster.

Our hope is that by performing this experiment, it will lead to further science and medical research on Vibrio Cholera, but also any bacteria that could be fought by a good virus, and the reactions in a micro gravitational setting. It could help improve prevention of disease caused by bacteria, such as Vibrio Cholera, as well as lead to further scientific research.

VI. List of Reference Publications

References

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Orlova, E. V. (2009, April 8). *National Centre for Biotechnology Information.* Retrieved October 20, 2016, from How viruses infect bacteria: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2670874/

Roman, D. (2016, Septembre 22). *Student Spaceflight Experiment Program*. (D. Roman, Producer, & National Center for Earth and Space Science Education) Retrieved Octobre 16, 2016, from SSEP Mission 11 to ISS Mini-Laboratory Operation: http://ssep.ncesse.org/current-flight-opportunities/ssep-mission-11-to-the-international-space-station-iss/ssep-mission-11-to-iss-mini-laboratory-operation/

Xu, H., Roberts, N., & Singleton, F. (1982, August). *Springer Link*. (Springer-Verlag, Producer) Retrieved Novembre 1, 2016, from Survival and viability of nonculturableEscherichia coli and Vibrio cholerae in the estuarine and marine environment: http://link.springer.com/article/10.1007/BF02010671

Zhang, S. (2014, April 10). *What Happens to Bacteria in Space?* (J. Cook, Ed.) Retrieved October 20, 2016, from Gizmodo.com: http://gizmodo.com/what-happens-to-bacteria-in-space-1559160714

VII. REQUIRED Letter of Certification by the Teacher Facilitator

Date: November 2016

I certify that the student team designed the experiment described herein and authored this proposal, and not a teacher, parent, or other adult. I recognize that the purpose of this letter is to ensure that there was no adult serving to lead experiment definition and design, or write the proposal, and thereby provide content and/or professional expertise beyond that expected of a student-designed and student-proposed experiment.

I also understand that NCESSE recognizes that facilitation of thinking across the student team through advice and counsel by the team's Teacher Facilitator, other teachers, and local area and national researchers, is not only to be encouraged but is absolutely vital if students are to receive the necessary guidance on the process of scientific inquiry, experimental design, how to do background research in relevant science disciplines, and on writing the proposal. I also understand that it is appropriate for the Teacher Facilitator and other teachers to provide editorial comment to the student team on their proposal drafts before proposal submission.

I also certify that the samples list and the special handling requests listed in this proposal are accurate and conform to the requirements for SSEP Mission 11 to ISS. I confirm that the team, after reviewing their procedure and budget for obtaining the samples for the experiment, is certain that they will be able to obtain the necessary samples for their experiment in time to meet the deadline for shipping the flight-ready FME to Nano Racks. If using human samples, the team is aware that these samples must be tested for prohibited viruses before the experiment can be selected for flight. Finally, the Teacher Facilitator certifies that the student team will have access to the proper facilities to prepare the FME mini-laboratory for flight and to analyze the samples after the flight.

Teacher signature :

Name: