Microgravity's Effect on the Virulence of Bacteriophage qB on *Escherichia coli* as a Possible Indicator of the Down-Regulation of Host Factor Hfq

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Introduction

The virulence of viruses is a topic of interest for the wellbeing of human health during space travel. Little research has been conducted on differing virulence between bacteriophages in space and on earth. However, prior research suggests evidence that a difference may exist. Hfq is an RNA binding global regulator protein present in *E. coli* which has been shown to be required for Coliphage qB to infect *E. coli*. In a squid-vibrio experiment conducted under simulated microgravity, the hfq protein production was seen to be down-regulated in *Vibrio fischeri* (Grant, 2014). Our experiment aims to imitate results found by Grant under a real microgravity situation in *Escherichia coli*, given that Hfq is highly conserved in bacteria. The experiment will compare the virulence of *Coliphage* qB on *E. coli* in space to the virulence on land. The resulting difference in rates of Coliphage qB infection of *E. coli* may hint at unknown molecular mechanisms that bacteria and/or viruses employ under the effects of microgravity, and may provide evidence to suggest that *hfq* is also down-regulated in *E. coli* in microgravity. The implications of the results found by this experiment will be a step into determining the virulence of viruses and other infectious organisms during spaceflight and may lead to effective preventative measures to safeguard the health of humans in space.

Experimental Rationale

The cells of *E. coli* will be counted with a hemocytometer. Lower numbers of *E. coli* mean that more Coliphage qB have infected the cells. The presence of Hfq is required for this infection. Thus, it can be determined if microgravity increases or decreases virulence relative to the control sample on earth. If microgravity causes Hfq to be expressed and translated less in the *E. coli* on the ISS than in the *E. coli* on earth, then Coliphage qB will have fewer host factors to carry out its infection and replication, thus more *E. coli* cells will be present. If the hypothesis is shown to be true, this may suggest that Hfq is indeed down-regulated in *E. coli* as it is in other bacterial species.

Experimental Materials

The materials proposed to be used in this experiment are:

Fluid Mixing Enclosure Mini Lab (Type 3): The FME Mini Lab is the approved closed system apparatus that will be used universally by all experiments to be conducted in the Student Spaceflights Experiment Programs (SSEP) Mission 12. Our experiment will be using Type 3 FME which contains 3 chambers separated by two clamps. The *E. coli* pellet, Coliphage qB culture, and 20% Formaldehyde solution will be kept isolated in the Type 3 FME tube, and will then be mixed during flight in subsequent steps. The FME Minilab will be provided by SSEP.

Escherichia coli pellet: This pellet will be used to maintain a stable culture of E. coli during the commute from University of Bridgeport to the International Space Station, at which point the Luria-Bertini Broth (known as "nutrient medium" hereafter) will be released to initiate the reaction. The pellet allows the experiment to be conducted with numerous bacteria while using only a fraction of the volume available in Type 3 FME Mini Lab. The bacteria in the pellet will be inactive until the nutrient medium is mixed with it. This helps complete two major constraints set by the SSEP proposal committee. Additionally, the specific strain of E. coli that will be used falls under Bio-Safety Level 1. The pellet will be obtained by from the University of Bridgeport.

Bacteriophage qB (Coliphage qB): This specific bacteriophage is proposed to be used because Guisbert et. al. has mentioned that the Hfq protein is the host factor that is necessary for the Bacteriophage qB to infect *E. coli* and replicate. The Bacteriophage qB will be purchased using funds from the University of Bridgeport.

Nutrient Medium: Luria-Bertani Broth (LB Broth): 1% bacteriological peptone, 0.5% yeast extract, and 0.5% NaCl. This nutrient medium is one of the most commonly used bacteriological growth media. The medium will be obtained by from the University of Bridgeport.

20% Formaldehyde: 20% Formaldehyde solution is proposed to be used as it follows the accepted guidelines for problematic samples set by the SSEP committee. This solution will be used to stop the reaction and inactivate the experiment by making the *E. coli* inert and sterile so that the gravity on the way back down to earth won't ruin the results. This 20% Formaldehyde solution will be obtained from the University of Bridgeport.

Hemocytometer: A hemocytometer is an apparatus that allows investigators to conduct a manual cell count, which will be used to count the number of cells of bacteria remaining when the FME tube returns from the ISS. This apparatus will be obtained from the University of Bridgeport.

Experimental Procedure

- An FME minilab will be used to conduct the bio-chemical reactions.
- The FME Minilab will be divided into 3 chambers using clamps, Chamber I will contain a bacterial pellet of E. coli; Chamber II will contain a viral culture of Coliphage qB with a nutrient medium; Chamber III will contain a solution of 20% Formaldehyde. Chamber I and Chamber II will be separated by Clamp A, while Chamber II and Chamber III will be separated by Clamp B.

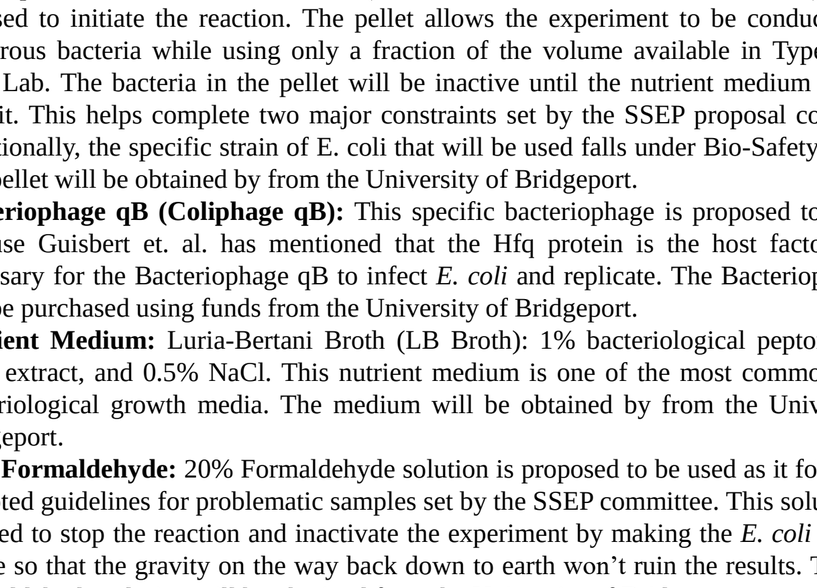
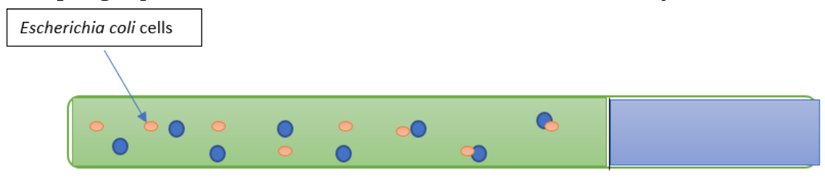
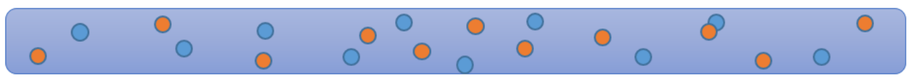


Figure 1: Fluid Mixing Enclosure Mini Lab Set-up

- The FME will be sent to ISS and the experiment will be initiated at day A+2 by unclamping Clamp A and shaking the tube gently for 30 seconds while Clamp B is still intact. This process will initiate interaction between the *E. coli* and the Coliphage qB. This condition will be maintained until day U-2.



- At day U-2, Clamp B will be unclamped which will release the 20% Formaldehyde solution to inhibit the reaction to maintain the states of the viral and bacterial cultures.
- Upon return to Earth, the FME Mini Lab will be refrigerated through its commute back to University of Bridgeport.



Procedure Testing

- A mock procedure testing was conducted using an apparatus that imitated the FME Mini Lab Type 3 at University of Bridgeport to determine if the volumes predicted were plausible. The procedure was carried out successfully and proved to be effective. Water was used as a supplement for the liquids to be used in the experiment. During testing, we concluded that the volume of Coliphage qB and LB Broth mixture had to be reduced from 5mL to 4mL to fit the specifications of the Mini Lab. The procedure was conducted by shaking the contents after releasing Clamp A, and then after Clamp B. Mixing was observed which proved that the procedure was effective and would comply with the experiment.

Experimental Analysis

- **Experimental System of Samples:** The FME Mini Lab that returns from the International Space Station will be collected at the University of Bridgeport. The *E. coli* sample present inside the FME Mini Lab will be extracted and put through a Hemocytometer manual cell count kit. The cells of *E. coli* bacteria will be counted by the co-investigators and recorded.
- **Control System of Samples:** The sample from the FME Mini Lab that was present at University of Bridgeport will be extracted and put through the Hemocytometer manual cell count kit. The co-investigators will count the number of *E. coli* bacteria cells.
- **Result Analysis**: The results found in the observations using the Hemocytometer in both experimental and control samples will be compared; initially the mass of the bacterial pellet was the same so the number of cells of bacteria are assumed to be approximately the same. The volume of Bacteriophage qB used in both the systems will be the same, so the number of viruses is assumed to be approximately the same. At the end of the experiment, if the number of bacteria is lower in the control tube and higher in the experimental tube, the hypothesis will be shown to be true. Alternatively, if the number of bacterial cells in the experimental procedure will be the same as in the control, we will accept the null hypothesis to be true. This will procure insight on the possible differential virulence upon bacteria under the effects of microgravity, and may lay the foundation for future studies in determining if Hfq is indeed the protein responsible for this difference.

Implications and Future Work

E. coli may have unknown molecular, genetic or biochemical mechanisms that would result in more *E. coli* cells being infected with Coliphage qB in space than compared to the ground sample. Therefore, we cannot assume that confirmation of the hypothesis directly infers that Hfq is downregulated; however, confirmation of the hypothesis will act as a "green light" for future research of bacteriophage virulence in *E. coli*. Verification that Hfq is indeed downregulated under the effects of microgravity may be achieved by comparing mutant *E. coli* strains with various gene knockouts, such as Δhfq, and by conducting quantitative reverse transcription PCR to determine if Hfq is in fact being fully translated.

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