**How Bacteria Handles a Bad Day**MICR 3333 – Spring 2019

**Abstract**

Bacteria have powerful responses to an assortment of stresses. Bacterial cells have developed their own stress responses to survive sudden, and extreme changes in conditions. In this micro-review, the model Gram-positive Bacterium *Bacillus subtilis’* response to stress was studied in the environment of human spaceflight. In an unlikely opportunity, scientists were afforded the opportunity to send cells on two separate missions to the International Space Station. This first spaceflight study of two separate missions using the same bacterial strain allowed scientists to compare mission-to-mission variability to the spaceflight environment. Cells were grown to late-exponential/early stationary phase, frozen, then returned to Earth for comparison with ground control samples. Microbial cell’s exposure to microgravity in spaceflight resulted in stress from changes in their environment, which caused the cells to respond with a complex set of stress responses known as “spaceflight syndrome.” Countless attempts have been expended to understand bacteria’s response during spaceflight. The comparison of data was challenging because cells had only been flown on single space flights. In this study, the effects of exposure to the spaceflight environment on the *Bacillus subtilis* is shown by identifying sets of genes commonly expressed in both missions to the International Space Station.

**Introduction**

What we refer to as “stress responses” are the changes in gene expression as bacteria adapt to changing or suboptimal environments (Gottesman, 2017). The human spaceflight environment is unlike other confined study environments because of the unique factor of reduced gravity (microgravity), which applies physiological effects on bacteria. Scientists have studied microorganisms in the spaceflight environment during focused research in the past, but have found it difficult to understand their responses to spaceflight stress and their underlying causes. Early studies only performed experiments on a small selection of Gram-negative bacteria under many different culture conditions with experimental variation derived from technical effects, or biological effects. In these early spaceflight experiments, the scientists tried to control the variation by using prior spaceflight replicates. However, the bacteria being studied had only flown on a single mission to space, so there was nothing to compare for variability (Morrison, 2019). There is stiff competition due to limited space for research on space missions, so new experiments generally get preference over an experiment previously done. Because of this preference and limited space on missions the study of how bacteria handle spaceflight was at a standstill.

**Recent Progress**

In 2015, scientists were afforded the opportunity to send an experimental package to the International Space Station to test the responses of the Gram-positive Bacterium *Bacillus subtilis* to the human spaceflight environment. This experiment was called BRIC-21 and a Canister-Petri Dish Fixation Unit was used as the hardware for transporting the bacteria. Cells were grown, frozen, and returned to earth for analysis of the growth, antibiotic resistance, frequency and spectrum of mutagenesis exhibited by flight samples in comparison to matched ground samples. Unlike the experiments in earlier studies, the same scientists had the unbelievable luck to fly a second mission (BRIC-23) to the International Space Station using the same *Bacillus subtilis* strain with the same hardware to perform the same analysis on the samples for comparative analysis of the *Bacillus subtilis* strain. In comparing the response of *Bacillus subtilis* cultures exposed in spaceflight environment RNA-seq was used to compare flight samples vs. matched ground samples.

**Discussion**

Scientists believed that the outcome of their experiment to analyze the effects of the stress of spaceflight on the *Bacillus subtilis* strain in two separate spaceflight experiments would identify genes that were significantly up-regulated, or significantly down-regulated in both space missions, and define genes whose response to stress were consistent in response to spaceflight environment stress.

The *Bacillus subtilis* strain 168 consists of 4397 total genes, of which 4280 encode proteins. Scientist reviewed the RNA-seq data from BRIC-21, which resulted in identifying 293 total genes whose expression differed in flight samples vs. ground samples. This represented approximately 6.8% of the total protein coding genome. Of these 293 total genes, 177 were higher in flight samples and 116 were higher in ground samples. In the identification process of the second flight BRIC-23, there were 255 total genes whose expression differed in flight samples vs. ground samples. This represented approximately 6% of the 255 total. Of these genes, 163 were higher in flight samples and 92 were higher in ground samples (Morrison, 2019). The scientist didn’t expect such a variation in between flight experiments because they had worked at keeping discrepancies between the two flights at a minimum. However, after studying the results of both studies and comparing the datasets obtained from both space missions, it was found that only about one-third of the genes responded to stress the same way in both space missions.

One of the ways genes were significantly up-regulated in both space missions was in biofilm formation. During both spaceflights, environmental conditions caused genes in both flights to produce biofilms. Bacterial biofilms have been the cause of water supply contamination in space habitats, and data from spaceflights have documented biofilms containing *Bacillus subtilis* in the Space Shuttle water system. While the water system of the BRIC-21 and BRIC-23 were not contaminated, there were numerous biofilm-related genes observed to be remarkably up-regulated in both flights.

*Bacillus subtilis* prefers an oxygen rich environment for optimum growth. In transcripts related to fermentation, anaerobic respiration and subtilosin production, this study showed an up-regulation in ground control samples (Morrison, 2019). However, in flight samples of transcripts associated with siderophore, production showed an up-regulation as well. Siderophores binds iron with high affinity for import and is produced by *Bacillus subtilis.* The problem with this being that flight samples and ground samples were experiencing different degrees of oxygen availability in the two experiments. Since *Bacillus subtilis* prefers an oxygen rich environment the best condition to grow, there needs to be better hardware to accommodate this need for more oxygen. A new piece of hardware is being designed and constructed of PDFU inserts that will maintain a constant geometry of liquid and air space.

Significantly up-regulated transcripts for biotin and arginine biosynthetic genes were found in flight samples. Biotin (Vitamin B) is important in fatty acid metabolism, and central metabolism. The amino acid arginine converts glutamate to citrulline. Convection ceases in microgravity and the transport of nutrients or waste products through liquid media becomes limited.

An up-regulated observation of a number of genes encoding resistance and toxic functions in flight samples might have first suggested to scientist that flight samples could be exhibiting a higher degree of resistance to antibiotics or toxic compounds. However, samples were exposed to 72 antibiotics and there is not a significant difference in resistance levels found in flight samples vs. ground samples (Morrison, 2019).

While the two experiments were treated as replicate experiments, scientists noted that one notable difference between the two space mission experiments was the difference in incubation times. The first flight’s incubation time was 25 hours, while the second flight was 36 hours. The hardware used, known as the Canister-Petri Dish Unit (BRIC-PDFU), did not allow for direct measurement of growth during spaceflight (Morrison, 2019). In future experiments, a possible solution to this hardware could be used to avoid the approximate 2/3 discrepancy in the two datasets.

**References**

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