Gene expression of pathogens in simulated microgravity

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Introduction

DNA damage due to radiation and bone loss are not the only factors that astronauts and future space travelers have to worry about when they are in space. There is strong evidence that astronauts become immunocompromised during spaceflight, and their weakened immune system makes them vulnerable to pathogenic bacteria that may have hitchhiked into the spacecraft. *Serratia marcescens* is a common opportunistic bacteria on Earth, and exists ubiquitously in wet environments. Because they are opportunistic pathogens, they pose greater risk for immunocompromised individuals. Previous experiments showed that *S. marcescens* grown on the International Space Station increased in virulence after injection into fruit flies on the ground (Fig 3). In this project, we used simulated microgravity (Fig 2.) to grow *S. marcescens* and used qPCR to measure expression levels of genes which may potentially be linked to the bacteria's increased virulence in microgravity conditions.

Methods

Bacteria Growth:

- 1st: LB+ 100 µg/mL streptomycin for 18 – 24 hours on a shaker.
- 2nd: dilution to A600 of 0.100 in LB, then further diluted 1:1000 in LB+100 µg/mL streptomycin.
- 10 mL of dilution was then poured into the rotating wall vessels.
- Removed all air bubbles and set the environment to 37 °C, rotation to 25 rpm[1], and duration for 24 hours.

Gene Expression Analysis:

- RNeasy mini kit from Qiagen
- cDNA was created using the BioRad iScript synthesis kit.
- qPCR was done using the BioRad SYBR Green Kit
- Fold change calculated using ΔΔCt
  - recA and rpoS used as housekeeping genes

Figure 1. Growth of different strains of *Serratia marcescens*, all containing varying levels of prodigiosin production (red pigment).

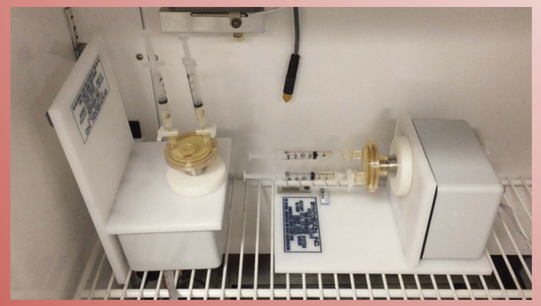

Figure 2. Rotating wall vessel (Synthecon) inside of a 37C incubator, used to grow bacteria. The vessel on the right is the simulated microgravity vessel, and left is the control vessel.

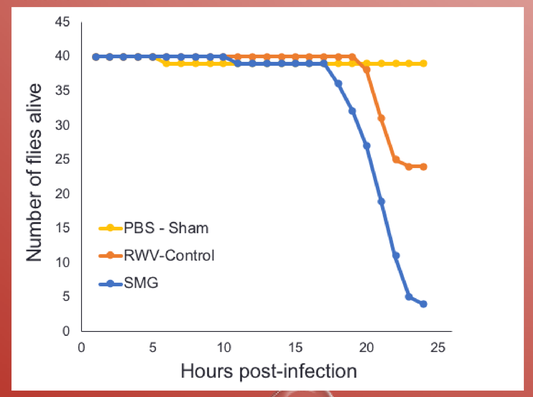

Figure 3. After 24 hours of growth in the RWV, *S. marcescens* is significantly more virulent to fruit flies when injected into the body cavity. The *in vivo* growth rate of the bacteria after injection is also higher than control (data not shown).

Results

Table 1. Fold change values for selected genes, SMG relative to RWV control. Descriptions are based on literature, but may have a different function in *Serratia marcescens*.

Gene Name	Fold Change	Description
asnB	28.84	asparagine synthetase B - Catalyzes the aspartate to asparagine, necessary for survive assault by the immune system
FlgG	2.09	Helps form the filaments of bacterial
secY	2.06	Essential for protein secretion across the membrane
FliE	2.05	Involved in biogenesis of flagella
nudE	2.00	Enzyme superfamily that helps remove metabolites and stress-induced signaling from the bacterial host
lpxD	2.04	Lipopolysaccharide biosynthesis gene, essential for biofilm formation, and expression reduced bacterial attachment airway epithelial cells
tatB	2.08	Reduced expression results in slowed cytochrome oxidase c activity, and susceptibility to intracellular infection.
hslU	2.31	Heat shock protein (ATPase) that is response to cell stress
groEL	1.21	Heat shock protein, involved with host cell
dnaK	2.00	Involved in the heat shock response, bacteria cell survival

Discussion

- In this project, we discovered genes which may play a role in the increase of virulence in *S. marcescens*.
- Some genes function in DNA repair and replication, and some play a role in the bacteria's motility.
- The gene asnB shows overexpression of 28.84 FC in SMG relative to the control. This gene is responsible for arginine catabolism, a mechanism that helps bacteria replicate once it's inside the host immune system[4].
- Because we see increased replication of SMG bacteria inside the fruit fly host after infection, this could be one of the genes responsible for increased virulence in microgravity conditions.
- Further study is needed to understand the bacteria's increased in virulence and the consequential risks.
- A future approach can include RNA sequencing of samples to get broader patterns of expression, longer growth times in SMG, and the comparison of these SMG results to spaceflown bacteria.

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References

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